Chapter 14

The Estimation of Species Richness in Pennsylvanian Coal Swamp Communities

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Introduction

One goal of an introductory biology course is to convey a sense of what biologists actually do for a living. Because science is a "hands-on" activity, a term paper, although valuable, seems inappropriate, while laboratory exercises are often too predictable. An independent research project that emphasizes observation, measurement, and critical thinking is the best way to simulate actual research. As a result, I attempt to involve all my freshmen non-majors in some form of research. Their research projects are based on fossils, because fossils can be studied safely outside of normal lab times. Little or no dangerous chemicals and/or equipment are required.

Every student in my non-majors, freshman biology course must prepare and identify a small fossil fish (*Knightia, Diplomystus,* or *Priscacara*) from the Eocene Green River Formation (Dolph and Dolph, 1990). This project is equivalent to a major term paper. After learning the required techniques in a regular laboratory session, the students complete their work at home. At the end of the semester, the students turn in a completed preparation and a short essay identifying their fish. Although my laboratory assistants are required to help with the fossil fish, I give the introductory instructions on appropriate technique to each lab class. I also stay in the lab to help any and all students get started on their fish. The project turns out to be a great way for elementary education majors to obtain demonstration material for their classes. What young child isn't fascinated by fossils?

My honors students choose from a number of different projects—Russian ammonites, larger Eocene fossil fish, Cambrian trilobites, dinosaur bone structure and diet, and the structure of Pennsylvanian coal swamp communities. These projects replace the normal laboratory exercises that the rest of the non-majors class are engaged in. The honors students select specimens from a range of fossils, research their material in the library, design a set of procedures that will allow them to study that material, and then spend the rest of the semester working on their project. At the end of the semester, they must turn in their specimens and a paper written in the format of a science journal. The honors students normally work in the back of the laboratory during their regularly scheduled lab, but much of their work proceeds through one-to-one discussions outside of class. In addition, I help them prepare difficult parts of their specimens, if they are concerned about how to carry out the proper technique or are simply afraid they will damage the material.

I have been able to use this approach because I am the only instructor who teaches non-majors biology (2 to 3 sections per semester). The approach would have to be modified for use in larger classes (more than 50 students), in classes having more than 3 sections, or in classes having more than one instructor.

My initial presentation at the ABLE workshop (9 June 1995) dealt with the reconstruction of the frond of *Karinopteris,* a Pennsylvanian seed fern (Dolph, *et al.,* 1994). The exercise on *Karinopteris* illustrates the most basic questions a paleobotanist can ask. These questions are the same for each of

the fossil groups my honors students might study. What types of organisms (e.g., seed ferns, ferns, etc.) are found in the sediments being studied? What did these organisms look like? These questions introduce the students to three fundamental aspects of research—pattern recognition (who's there), counting (rare or common) and measuring (how big), and reconstruction (what parts go together and why). Once the students have a feel for the organisms that lived in the area where the sediments were deposited, they can go on to more challenging questions. What was the paleoenvironment like? What types of communities were present? How many species grew there?

In this paper, I would like to explore a new method for estimating the number of species that grew at any one locality in a coal swamp. Originally, I thought that I could use the flora at Roaring Creek, the locality of *Karinopteris* and the Indiana paper coal, as the example. However, the depositional history at Roaring Creek is more complex than I can cover here (Dolph, work in progress). The example I will discuss is from the Little Coalburg coal of West Virginia (Kosanke, 1988; Dolph, work in progress).

As with many studies in paleontology, the approach introduced here is not nearly as rigorous as an experiment in molecular biology. Rather than writing a detailed lab exercise on a specific coal, I will try to give an overview based on how one of my honors students might view a project dealing with species richness in any Pennsylvanian coal. You should adapt this exercise to material that is locally available. Tailoring the lab to a specific coal would be impractical, if you did not want to come to Indiana to collect your specimens. What would happen if the mine closed? You might also find it easier to develop a lab based on Devonian microfossils or Cretaceous coals from the West.

Species Richness

When discussing the flora of the Pennsylvanian coal swamps, one of the more difficult estimates to reach is the number of species that grew at a locality. Species diversity (more properly called species richness (McIntosh, 1967)) could be estimated using either the gray shale macrofossils, a paper coal, a suite of coal balls, or a series of palynological samples.

The first two sources are not likely to yield an accurate estimate of species richness. The macrofossils obtained from the gray shale are too bulky to allow a truly representative sample to be collected, and they are sufficiently fragmentary that paleobotanists cannot be absolutely certain how many reconstructed plant species might be found in the flora. The fear that some specimens might have been transported long distances in water coupled with the possibility that other forms could be destroyed during transport and burial adds further uncertainty to the number of species that might have been present. However, sufficient information has been collected to indicate that the flora characteristic of these clastic-dominated environments was quite different from that characteristic of the coal swamps (Willard, 1989b).

Paper coal is a descriptive term referring to the appearance of weathered, cuticle-rich coal. The coals are largely vitrain with an abundant layered cuticular component. Because cuticle is a biopolymer which is highly resistant to decay, it can be selectively concentrated in subaerially exposed plant litter (peat). At Roaring Creek, the peat that lithified into coal must have been exposed to the air at isolated sites within the original coal swamp, resulting in the formation of a paper coal composed almost entirely of the fronds of the seed fern *Karinopteris* (Dolph, *et al.,* 1994). The concentration of *Karinopteris* does not indicate that it formed a monotonous stand in the area. *Karinopteris* simply had a cuticle that was thick enough to resist decay during exposure, while the cuticles of other plants were destroyed. The method by which paper coal forms results in the progressive destruction of the thinner plant cuticles and precludes the possibility of using the flora of the paper coal to determine species richness, because the estimated number of species present will always be too low.

DiMichele and Phillips (1988) calculated the relative percent volume (a measure of biomass) for the taxa found in the Herrin (No. 6) coal member of the Old Ben Mine in Illinois using coal balls. They analyzed 634 coal balls having a total surface area of 28,236 cm^2. Their study is both monumental and impressive. The parent vegetation was inferred from the root systems and litter preserved in the coal balls. A complete sampling of plants from the ground layer to the canopy was theoretically possible. However, the fragmentary nature of the preservation in the coal balls did not allow them to reach definite conclusions about the number of species present in any of the strata. While some species could be identified (e.g., *Lepidophloios hallii*), most comparisons were made at the level of the major groups represented (e.g., ferns or seed ferns). The biomass comparisons of DiMichele and Phillips (1988) were therefore between major groups and not between specific plants. No estimate of species richness was possible. This approach (e.g., DiMichele and Phillips, 1994) is not applicable to all sites, because coal balls are rare to absent in most coal beds (Cecil, 1990).

A numerical estimate of species richness can be obtained by sampling a coal for pollen and spores. Not all paleobotanists would agree with this statement. Wilson Stewart and Gar Rothwell (1993: 164) in their excellent and very readable paleobotany textbook state "...that one cannot use the dispersed spore context of a locality in a coal swamp to determine the number and kinds of plants that made up the vegetation of that specific depositional site." This argument is advanced because some pollen and spores types might have been transported long distances before being deposited. Therefore, plants that did not live in the coal swamp might be counted and species richness overestimated. Similar arguments have not prevented Quaternary palynologists from preparing detailed analyses of the vegetation detected in younger sediments. Care must simply be taken when analyzing the data.

How far could transport mechanisms carry pollen and spores in the coal swamp? The dominant plants (lycopods) of the tropical coal swamps of the Pennsylvanian (Westphalian A–D) were evergreen, had a basic pole architecture, and were widely dispersed without a closed canopy. In contrast to lycopods from clastic-dominated environments, they were shorter (deduced from basal trunk diameters of 14–35 cm) and had a short lifespan (10 years or less)(DiMichele and Phillips, 1994). The amount of air movement and associated pollen and spore transport through such a forest could have been great. Plants more characteristic of clastic-dominated environments whose spores and pollen might have been transported into the wetter parts of the swamp could include the lycopods *Paralycopodites* (*Lycospora micropapillata*), *Sigillaria* (*Crassispora kosankei,* its microspore, and *Laevigatosporites glabratus,* its megaspore), and *Chaloneria* (*Endosporites globiformis,* its microspore, and *Valvisisporites auritus,* its megaspore), the tree fern *Psaronius* (*Laevigatosporites globosus*), the medullosan *Sutcliffia* (*Punctatisporites kankakeensis*), and the cordaitean *Pennsylvanioxylon* (*Florinites*). Since the coal was deposited in standing water, fluvial transport might also contribute to long-distance transport.

The dominant plants at the site and rarer plants that were prolific spore producers should leave abundant pollen and spores. However, do rare grains indicate long-distance transport or simply a lower species density, poor spore production, or a restricted habitat for their producers? Studies of *in situ* stumps indicate that both tree density and stand composition varied widely in response to local environment in the coal swamps (Gastaldo, 1986a, b; DiMichele and DeMaris, 1987; DiMichele and Nelson, 1989; Wnuk and Pfefferkorn, 1987). The plants were distributed along gradients defined by edaphic conditions (standing water to exposed peat), nutrients (low to high), and clastic influx (none to high). The swamp was a mosaic of habitat patches, and the distribution of many plants was discontinuous and highly clumped (DiMichele and Phillips, 1994). If a mosaic of habitats was present, the spores representing plants from clastic environments might not have been transported any great distance at all.

Do the pollen and spore floras overestimate species richness? The species richness estimates reported in this paper are well below those found in modern tropical forests. A mangrove swamp (*Rhizophora mangle*) and a *Mora* swamp sampled on the west coast of Costa Rica had only one and seven species of trees and shrubs, respectively (Holdridge, *et al.,* 1971). These values increase to 306 species of trees and shrubs in a 50-ha, old forest plot on Barro Colorado Island (Foster and Hubbell, 1990), 570 species from La Selva on the west coast of Costa Rica (Hammel, 1990), 690 species in the Manu floodplain forest in southeastern Peru (Foster, 1990), and 745 species from the Reserva Ducke in the central Amazon Basin (Prance, 1990). Even allowing for major taxonomic, structural, and functional differences between today's angiosperm-dominated tropical forests and the pteridophyte-dominated forests of the Pennsylvanian (an exception would be cordaite (gymnosperm) abundance in Westphalian B and C (DiMichele and Phillips, 1994)), a coal swamp flora of 69 plants (see below) representing a mosaic of local habitats does not seem to be a wild overestimation of the number of plant species that might have been growing in the immediate vicinity.

The dominant arborescent species in the coal swamp are always abundantly represented (e.g., *Lycospora* and *Laevigatosporites*), but the richness of the sample is determined by plants that make up only a small portion of the biomass. If the coal swamps increase in richness through geologic time by adding species to a lepidodendroid-dominated framework (DiMichele and Phillips, 1994), some method of estimating richness, the number of species present, must be developed or the *amount* of increase will remain a matter of speculation.

The greatest drawback in estimating species richness through pollen and spores has been the lack of an acceptable technique to make the estimate. A simple count of the species of pollen and spores found gives the lowest possible estimate of richness. How many species did we miss? Recent advances in ecological methodology which allow researchers to estimate species richness in unseen algal populations which are not amenable to complete census also hold promise in palynology where a similar widely dispersed and unseen community is hidden not in water but in coal.

Materials

Coal (one 2 cm square lump or 50 g per student)
Concentrated nitric acid (400 ml)
Saturated potassium chlorate solution (200 ml)
Sodium hydroxide solution, 10% (800 ml)
Alcohol dehydration series, 25%, 50%, and 95%
Glycerine and ethyl alcohol, 1:1 mixture (800 ml)
Glycerine jelly
Sieve, 65-mesh
Ehrlenmeyer flasks, 250-ml (1 per student)
Centrifuge tubes, 100-ml (1 per student)
Rubber stoppers (1 per student)
Glass stirring rods (1 per student)
Disposable pipets
Mortar and pestle
pH paper
Hot plates
Microscope slides and cover slips

Selecting and Processing a Coal Sample

The students should analyze a coal seam (or other microfossil source) close to their campus. The appropriate references to the name of the coal, its age, and a listing of the pollen and spores isolated from the coal can be obtained by contacting your State Geological Survey or your institution's Geology Department. Kremp's (1968) *Morphologic Encyclopedia of Palynology* is an excellent introduction to the terminology used to describe pollen and spores. Ravn's (1986) work on the coals of Iowa is an excellent source for photomicrographs of the pollen and spores characteristic of the Pennsylvanian. Stewart and Rothwell (1993) have produced a very readable text, *Paleobotany and the Evolution of Plants,* that can be used to introduce students to the types of plants that inhabited the coal swamps. DiMichele and Phillips (1994) have drawn on their vast experience with Pennsylvanian plants to discuss coals, coal swamps, and coal swamp habitats.

To be identified, the pollen and spores must be released from the coal. A number of different approaches are possible (Doher, 1980). The following procedure, which takes about two weeks to complete, is simply one of several options:

1. Using a mortar and pestle, crush a small lump of coal (about 2 cm square) and place the remains in a 250-ml Ehrlenmeyer flask. Wash and dry the mortar and pestle very carefully between uses. Every student should have a separate set of glassware to prevent contamination of their sample with pollen and spores from other coals. Remember that it is the unique species (see below) that determine the final estimate of species richness.
2. Prepare 600 ml of Schulze's Reagent by adding 400 ml of concentrated nitric acid (HNO_3) to 200 ml of a saturated solution of potassium chlorate ($KClO_3$) in a fume hood while wearing rubber gloves, an apron, and goggles.
3. Slowly add Schulze's Reagent to the coal in a fume hood. Some minerals (chiefly carbonates) will react violently with this reagent. *CAUTION***:** The presence of pyrite may cause an explosion when Schulze's Reagent is added to the coal. Proceed very slowly. Have a beaker of cold water available to dilute the mixture, if necessary.
4. Mix the coal and the macerating agent using a glass stirring rod and allow the mixture to stand in the fume hood. Some coals (bituminous coals) will macerate in 1–3 days while others (anthracites) may take longer than a week.
5. Examine the mixture periodically by removing a small sample with a disposable pipet and looking for microfossils under the microscope. If free microfossils are present, the maceration is nearing completion. At this time, the coal should form a uniform sludge on the bottom of the flask.
6. Decant the macerating agent taking care that the sediment at the bottom of the flask is not disturbed.
7. Fill the flask with distilled water and allow the sediment to settle out. Repeat this procedure until the water tests neutral with pH paper.
8. After the last washing, replace the distilled water with 10% sodium hydroxide (NaOH). The solution will turn dark brown, indicating that the vitrain is being removed. (Potassium hydroxide (KOH) may also be used. Reaction times are much faster, and the danger of destroying the pollen and spores is much greater.)
9. After about 8–10 hours, remove a small amount of sludge and check for free microfossils. If microfossils are observed, commence washing the sludge with distilled water until the solution becomes clear. Use pH paper to check for neutrality.
10. Screen the sample by washing it through a 65-mesh sieve. The > 65-mesh residue may be stored in 95% methanol, if you desire to check for megaspores. Wash and dry the screen very carefully between samples.

11. Decant the < 65-mesh residue into a 100-ml centrifuge tube. Centrifuge and decant. Continue until all the residue has been processed.
12. At this point, the spores may be stained using safranin or mounted directly.
13. Dehydrate the spores using an alcohol series consisting of 25%, 50%, and 95% alcohol (two changes). End the series with a 1:1 mixture of glycerine and ethyl alcohol.
14. The spores may be stored indefinitely in this mixture, or they may be mounted on slides in glycerine jelly.

None of these reagents should be disposed of by pouring them down the sink. They should be disposed of as required by your institution's chemical hygiene and safety plan.

Two different approaches could be used to set up the laboratory exercise. My honors students would carry out the entire procedure from maceration through slide preparation to identification, processing no fewer than 6 samples in order to have enough data to estimate species richness.

The second approach would be to prepare the slides yourself and then have your class(es) analyze the material. A class of twenty students could analyze two localities in a single laboratory period. Ten students would analyze ten samples from one locality (one sample per student and not one sample analyzed by ten students), and the other ten students would analyze samples from a second locality. This is not an exercise in counting pollen grains. Each student would scan the slides in his/her sample and prepare a species list. The final estimate of species richness would be made after pooling the data. The technique is robust enough that some misidentifications will not affect the final results based on the pooled data. The only way the results could be skewed would be by having one student insist on identifying a large number of exotic grains. If you have already studied the slides, you should be able to pick up this problem quite easily.

A number of factors might negatively influence your students' interpretations if they are unaware of their effects (DiMichele and Phillips, 1994). First, species producing both mega- and microspores should be counted only once if both spores are present. *Sigillaria approximata* produced both microspores (*Crassispora kosankei*) and megaspores (*Laevigatosporites glabratus*). Second, some spore taxa were produced by more than one species. *Laevigatosporites globosus* was produced by four different species of *Psaronius.* Third, some spore genera may represent either the developmental stages of a single species or the modified products of transport and deposition. All calamite cones are believed to have produced spores of the genus *Elaterites. Calamospora* and *Vestispora* may be the developmental stages of *Elaterites,* or they might represent this spore after it lost its elators. Finally, most palynological studies limit the size of the spores studied to 200 μm in diameter or less. This eliminates large prepollen grains such as *Schopfipollenites* and *Monoletes,* resulting in an underrepresentation of the medullosans. However, this sieve size also removes large megaspores, such as *Laevigatosporites glabratus* or *Valvisisporites auritus,* preventing some species from being counted twice if their microspores were also present. One factor that is not a problem is the differential production of spores between species. The jackknife method is based on the number of spore taxa found and not on their relative abundance.

Estimation of Species Richness

Because the community represented by a coal sample is indefinitely large and not amenable to complete census without sampling error, species richness must be estimated using a sample of individuals. A nonparametric approach, the jackknife, can be used to estimate species richness (Heltshe and Forrester, 1983). This approach assumes a random distribution of sample points and not a random distribution of species, the members of which might well be clumped. Only the presence (x) or absence (–) of a species is recorded at several sample sites (Table 14.1). The total number of species at a site is estimated using the total number of species present augmented by a

fraction of the total number of species that were encountered in one and only one sample (the unique species). Unique species are spatially rare due to clustering, but they are not necessarily numerically rare when found.

Table 14.1. Presence (x)/absence (–) data for the pollen and spores found at six collecting sites in the Little Coalburg coal of West Virginia (from Kosanke, 1988).

Taxon	Samples 1	2	3	4	5	6	Total
Acanthotriletes sp.	x	–	x	–	x	–	3
Alatisporites sp.	–	–	–	**x**	–	–	**1**
Anapiculatisporites spinosus	x	x	–	–	–	–	2
Apiculatisporis sp.	**x**	–	–	–	–	–	**1**
Calamospora breviradiata	–	–	x	–	–	x	2
C. mutabilis	–	–	**x**	–	–	–	**1**
C. parva	–	–	–	**x**	–	–	**1**
C. sp.	x	–	x	–	–	–	2
Cirratriradites sp.	x	–	x	x	–	x	4
Crassispora kosankei	x	x	–	–	–	–	2
Cyclogranisporites cf. *C. minutus*	–	–	**x**	–	–	–	**1**
C. sp.	–	–	**x**	–	–	–	**1**
Densosporites annulatus	x	x	–	–	–	x	3
D. triangularis	–	**x**	–	–	–	–	**1**
D. sp.	x	x	–	–	–	–	2
Dictyotriletes bireticulatus	x	x	–	–	x	–	3
D. castaneaeformis	–	**x**	–	–	–	–	**1**
D. falsus	–	–	**x**	–	–	–	**1**
Endosporites globiformis	x	–	x	–	–	–	2
E. sp.	x	–	–	–	x	x	3
Florinites antiquus	x	x	x	x	x	x	6
F. cf. *F. millotti*	x	x	x	–	–	–	3
Granulatisporites adnatoides	–	–	–	–	–	**x**	**1**
G. pallidus	x	–	x	–	x	–	3
G. spp.	x	x	x	–	x	x	5
Knoxisporites sp.	–	–	–	**x**	–	–	**1**
Laevigatosporites desmoinensis	x	x	x	–	x	x	5
L. globosus	–	x	x	–	x	–	3
L. latus	x	x	x	–	x	x	5
L. medius	x	x	x	x	x	x	6
L. minutus	x	x	x	–	x	x	5
L. ovalis	x	x	x	x	x	x	6
L. punctatus	–	**x**	–	–	–	–	**1**
L. vulgaris	x	–	x	–	x	–	3
Leiotriletes priddyi	x	–	x	–	–	–	2
Lophotriletes sp.	x	x	–	x	–	–	3
Lycospora granulata	x	x	x	–	x	x	5
L. micropapillata	x	x	x	–	x	x	5
L. pellucida	x	x	x	x	–	x	5

Table 14.1 (continued).

Taxon	Samples 1	2	3	4	5	6	Total
L. punctata	x	x	x	x	x	x	6
L. pusilla	x	x	x	x	–	x	5
L. sp. 5	–	–	–	**x**	–	–	**1**
L. spp.	x	x	x	x	x	x	6
Punctatisporites sp.	x	–	–	x	x	x	4
Radiizonates cf. *R. rotatus*	x	x	x	–	–	x	4
R. sp.	x	x	–	–	–	–	2
Triquitrites sculptilis	x	x	–	–	–	–	2
T. sp.	–	x	x	–	–	–	2
Verrucosisporites sp.	**x**	–	–	–	–	–	**1**
Vestispora cf. *V. costata*	**x**	–	–	–	–	–	**1**
V. fenestrata	x	–	–	–	–	x	2
V. sp.	x	–	–	x	–	x	3
Wilsonites delicata	–	–	x	–	–	x	2
W. sp.	–	x	–	–	x	x	3
Monosaccate	–	–	x	–	x	x	3
Unassigned	–	–	–	x	x	–	2
j	3	3	4	4	0	1	
k	3 +	3 +	4 +	4 +	0 +	1 =	**15**
s							56

Species richness is expressed as:

$$\hat{S} = s + ((n - 1) / n)\ k \qquad (1)$$

where $\hat{S}$ is the estimate of species richness, s is the total number of species in the sample, n is the number of samples, and k is the number of unique species. This approach has been used to estimate species richness in forest stands and benthic algal communities sampled by random quadrats. The technique should also work for equal volumes or weights of coal collected at random intervals along the face of a coal seam.

Pollen and spores are amenable to jackknife analysis for three reasons. First, they are often preserved when no other plant material remains because of their highly resistant walls (sporoderms) composed of sporopollenin. Therefore, the microfossils are more representative of the total flora than the macrofossils. Second, because they are smaller and lighter than macrofossils, a better mix of species is encountered in a sample of coal than could be expected in even a much larger collection of macrofossils. Finally, floras of the same age from different geographic localities or floras of different ages from strata at the same or different localities could be compared quite easily using this approach. Differences in the number of species present might reflect environmental change, evolution, immigration/emigration, extinction, or a combination of these factors.

The variance of the estimate of species richness is calculated as:

$$\text{var}(\hat{S}) = ((n-1)/n)\left(\sum_{j=1}^{s} j^2 f_j - (k^2/n)\right) \quad (2)$$

where var($\hat{S}$) is the variance of $\hat{S}$, f_j is the number of quadrats containing j unique species (j = 1, 2, 3, ..., s), n is the number of samples, and k is the number of unique species.

The confidence interval is calculated as:

$$\hat{S} \pm t_\alpha (\text{var}(\hat{S}))^H \quad (3)$$

where $\hat{S}$ is the number of species obtained using equation 1, t_α is the Student's t value for $n - 1$ degrees of freedom for the appropriate value of α (usually 0.05), and var($\hat{S}$) is the variance of $\hat{S}$ obtained using equation 2.

The jackknife tends to overestimate the actual species diversity, but the overestimate is always less than the underestimate given by a simple count of the species found. The results are unreliable if a community having a large number of rare species is sampled or if too few samples are taken in a highly diverse community. Insufficient sampling is the chief problem encountered when working with coal samples.

Sample Calculations

The calculation of species richness will be demonstrated using six samples from the Little Coalburg coal of West Virginia (Table 1; Kosanke, 1988). The three figures used in this calculation are obtained directly from Table 14.1—the total number of species (s) found in a given coal (for the Little Coalburg coal, s = 56), the total number of unique species (k) found (for the Little Coalburg coal, k = 15), and the total number of samples (n) taken (for the Little Coalburg coal, n = 6).

Substituting in equation 1:

$$\hat{S} = 56 + ((6-1)/6)(15) = 56 + (75/6) = 56 + 12.5 = 68.5 = 69$$

The variance is calculated after completing the following table:

Number of Unique Species (j)	Number of Samples with j Unique Species (f_j)
1	
2	
etc.	

Table 14.1 is scanned to find the number of samples having only 1 unique species, 2 unique species, 3 unique species,..., j unique species. For the Little Coalburg coal, the unique species are indicated in boldface (Table 14.1). The total number of unique species highlighted in the rightmost column (i.e., those found only at one sample site; k = 15) should equal the sum of the number of unique species in each sample column (3 + 3 + 4 + 4 + 0 + 1 = 15). The completed table reads:

Number of Unique Species (j)	Number of Samples with j Unique Species (f_j)
1	1
3	2
4	2

Substituting these values into equation 2, the variance (var($\hat{S}$)) is calculated as:

$$\text{var}(\hat{S}) = ((6-1)/6)[(1)^2(1)+(3)^2(2)+(4)^2(2)-((15)^2/6)]$$

$$= (5/6)(1 + 18 + 32 - 37.5) = (5/6)(13.5) = 11.25$$

For the 95% confidence limits ($\alpha = 0.05$), $t_\alpha = 2.571$ (Rohlf and Sokal, 1969; Table Q). Substituting in equation 3:

$$69 \pm (2.571)(11.25)^{1/2} = 69 \pm (2.571)(3.354) = 69 \pm 8.62 = 69 \pm 9$$

The species richness for the Little Coalburg coal is 69 ± 9 species. The lower confidence limit is 60 species, and the upper is 78. This assertion can be made with 95% confidence (i.e., there is a 5% chance that the statement is wrong).

The confidence limits around the estimate of species richness should decrease, if the number of samples analyzed is increased. The decrease is realized only if the pollen and spore macerates at each site are sampled with the same intensity. For example, the species richness for the Eagle coal (middle bench) of West Virginia was 64 ± 19 species (Kosanke, 1988; Dolph, work in progress). Of the six samples analyzed from the Eagle coal, one had 9 unique species while two others had none. Twenty-six microfossils were identified in first sample, but only 6 and 8 were found in the last two. Unless the last two samples were nearly barren (i.e., lacking pollen and spores), sampling intensity differed between the three samples, resulting in one of the samples having too high a number of unique species. Nevertheless, the Little Coalburg and Eagle coals both had about the same number of species (69 and 64, respectfully).

Theoretical Considerations

Would the number of species at a typical locality in the Pennsylvanian coal swamp support the same number of species through geologic time? Would contemporaneous localities at different points in the coal swamp support the same number of species? An analysis of the first question is currently underway for the coals of West Virginia (Dolph, work in progress). This work will not answer the second question, however. At the present time, a possible answer can only be hinted at by comparing the Little Coalburg coal with the three coals at Roaring Creek (bear in mind that Roaring Creek is older and in a different depositional basin than the Little Coalburg coal).

The species richness of the three coals at Roaring Creek was considerably higher than the species richness in the Little Coalburg coal. The number of species estimated for Coal A was 127; for Coal B, 97; and for Coal C, 95. Calculating confidence limits on these samples was not profitable, because only two samples were analyzed for each coal (Peppers, 1982). Why was the number of species found at Roaring Creek so much higher than the number of species found in West Virginia? The coals sampled at Roaring Creek are situated at an ecotonal boundary between swamp and upland

vegetation. Therefore, a higher number of species should be encountered at Roaring Creek because microfossils from more than one community are present in the sample.

How sharp was the ecotonal boundary at Roaring Creek? To answer this question, we must know the answer to a more global question. Were the communities of the coal swamps open communities, closed communities, or a mixture of both? In closed communities, the species are grouped into distinct associations which are separated by a sharp, ecotonal boundary. The richness at the boundary (possibly Roaring Creek) would be higher than in either of the communities that formed the boundary. In open communities, the species gradually move into and fade out of the vegetation along an environmental gradient. No clear boundary between vegetation types exists.

DiMichele (1994; DiMichele and Phillips, 1994) favors the closed community model. The compression/impression flora of the clastic-dominated upland environment (gray shales) is completely distinct from the flora of the peat-dominated lowland environment because the low pH, long periods of flooding or a high water table, and low nutrient levels clearly separated the coal swamp and its flora from the more equitable uplands and its flora. Compression/impression floras have fewer taxa, fewer specimens, and an overrepresentation of medullosan pteridosperms, ferns, and sphenopsids.

In their reconstruction the Middle Pennsylvanian Herrin coal swamp of Illinois, DiMichele and Phillips (1988, 1994) recognized the presence of several plant associations. One high-dominance, low-diversity association characteristic of standing water was dominated by *Lepidophloios hallii.* A second association was dominated by *Diaphorodendron scleroticum* and *Synchysidendron dicentricum* and grew on exposed to partially submerged peat. Diversity was high, and ground cover was present. The last association occurred on exposed peat near areas of clastic input. This community was dominated by *Medullosa,* a seed fern. The medullosans were concentrated in mineral-rich areas, and some grew in dense stands, which commonly caught fire. *Psaronius* tree ferns were common in the last two associations but not in the first due to persistent water cover that prevented reproduction. Tree ferns, sphenopsids, and seed ferns grew on levees and in clastic swamps. Cordaites were rare, probably because swamp conditions were unfavorable to these slow-growing, long-lived plants. However, *Pennsylvanioxylon birame,* a cordaite whose growth habit resembled the modern mangrove, grew in brackish to near-marine water.

Care must be exercised here because DiMichele's (1994) definition of a paleocommunity is most similar to the modern association. A coal swamp association defined in this manner did exist and was common for many millions of years. Recognition of the coal swamp association does not provide an answer to how community structure within that association changed at the local level through time. Not every stand in the modern beech-maple association is the same. Not every coal swamp community should be.

Can the associations recognized by DiMichele and Phillips (1994) be recognized in the palynoflora? Superficially, the answer is "yes." For the Little Coalburg coal (Table 1; Stewart and Rothwell, 1993), *Lepidodendron* and *Lepidophloios* (arborescent lycopods) are represented by *Lycospora. Diaphorodendron* is represented by *Crassispora.* Marattialean tree ferns are represented by *Punctatisporites* and *Laevigatosporites.* The isoetalean lycopods are represented by *Endosporites,* the cordaites by *Florinites,* and the calamites/sphenophylls by *Calamospora.*

At the level of specific species, the answer is more clouded. The task of tracing the affinities of microfossil species back to the specific plants that bore them has just begun. The spore genus *Lycospora* can be used to illustrate this point. Three spores encountered in the Little Coalburg coal (Table 14.1) are associated with specific species of *Lepidophloios* and *Lepidodendron.* Both of these genera were common in areas of standing water, but *Lepidodendron* preferred areas with a higher nutrient influx (DiMichele and Phillips, 1994). *Lycospora granulata* was produced by *Lepidophloios hallii*; *Lycospora pellucida* by *Lepidophloios harcourtii*; and *Lycospora pusilla* by *Lepidodendron*

hickii (Willard, 1989a). In addition, *Lycospora punctata* has been found in the cone species *Lepidostrobus* cf. *squarrosus,* which was borne on an unknown clastic-centered lycopod (Willard, 1989b). The affinities of the remaining three species of *Lycospora* found in the Little Coalburg coal are unknown. This fact is not surprising considering that the affinities of most of the more than 96 recognized species and varieties of *Lycospora* are unknown (Willard, 1989a). Other relationships are even more interesting. Although *Crassispora* is associated with *Diaphorodendron,* a lycopod, *Crassispora kosankei* was found in *Mazocarpon oedipternum* (Courvoisier and Phillips, 1975), the cone type of *Sigillaria approximata* (Stewart and Rothwell, 1993), a completely different type of lycopod. Although *Laevigatosporites* is characteristic of the marattialean tree ferns (e.g., *L. globosus* from *Psaronius*), *Laevigatosporites medius* was found in *Bowmanites bifurcatus,* a sphenopsid cone (Courvoisier and Phillips, 1975).

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