Transactions of the Illinois State Academy of Science
(1999), Volume 92, 1 and 2, pp. 15-22

received 1/30/98
accepted 8/2098

Allopatric Spore Ratio Variation in *Leucoagaricus hortensis* (Agaricales, Basidiomycetes), a Species New to Illinois

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ABSTRACT

Basidiospores from two populations of *Leucoagaricus hortensis*, one from Florida and the other from Illinois, were compared as to their average width relative to length. Statistical analysis demonstrated a significant difference between the two populations. Spores from the Illinois locale tended to be proportionately broader than those from Florida. Considering the climatic contrast between these two locales, this difference may reflect an adaptation to environmental factors, such as winter desiccation and cold. This is the first report of this species from Illinois.

INTRODUCTION

Basidiospore length, width, and length/width ratio are among the important microscopic features used for taxonomic identification of basidiomycetes (Singer, 1986). Because variation occurs between individuals and populations, however, problems of systematic assessment sometimes arise. The present study examines the degree and manner of such variation between two populations of *Leucoagaricus hortensis* (Murrill) Pegler, one from Illinois and the other from Florida.

Leucoagaricus hortensis is a saprobic agaric typically found in pasture habitats of warmer latitudes, sometimes occurring directly on manure of herbivorous livestock such as cattle and horses (Akers, 1997). Its basidiospores are ellipsoidal to ovoid in shape, and measure 7.3-13.5 x 5.8-8.5 μm (Akers, 1997). Originally described from Alabama by Murrill (1914), it was later described from Florida by the same author as three new species, *Lepiota humei*, *L. mammillata*, and *L. subfulvidisca* (Murrill, 1943; Akers and Sundberg, 1997). Since then it has been reported from the Caribbean (Dennis, 1952; Pegler, 1983; Rodriguez Gallart, 1990), Colombia (Franco, 1994), Texas (Metzler and

Metzler, 1992), and Hawaii (Don E. Hemmes, pers. comm.). In 1996, a collection was made in Jackson County, Illinois (SIUC Akers 96/9-25/001). This was the first collection of this species in the state, and apparently represents the northern limit of its known distribution.

In agaric species, sexual reproduction occurs when fertile, haploid hyphae of opposite mating types function as gametes by undergoing somatogamy (Moore-Landecker, 1990). Assuming that fertile hyphae in Illinois would have virtually no chance of mating with their counterparts from Florida because of the distance separating them, specimens from these two locales can be theoretically regarded as representing geographically separate populations (Hartl, 1988). The collection from Illinois thus enabled a study as to whether spore width would vary in relation to length differently between a southern population and a more northern one.

MATERIALS AND METHODS

Spore measurements were made by optical microscopy at 1000X using a calibrated ocular micrometer. For analytic purposes, spore width was designated as the criterion variable (Y). Its regression on two predictor variables (X), length (a continuous variable) and locale (a categorical variable), was studied. The specific goals were to discover (a) whether width could be predicted from length, or locale, or both, (b) whether the relationship of length to width differed between the two populations, and (c) whether there was any interaction between the two X variables. The latter proposition was tested using effect coding to determine the best predictive model. All statistical analyses were conducted using SAS (1989). Statistical significance was tested for by calculation of the F ratio, which is interpreted from a table of critical values dependent upon degrees of freedom, defined as n - 1. This type of test thus takes sample size into account as part of the calculation of statistical significance, in effect alleviating theoretical concern about whether a sample size is large enough to warrant the conclusions drawn (Hinkle et al., 1994).

Spore measurement data for the Illinois population were obtained by random sampling of spores from a lamella of the Illinois collection, which consisted of three basidiomata fruiting within several centimeters of each other. Six collections were used to represent the Florida population. One of these collections (SIUC WJS VIII-10-85-B2) was obtained by one of the authors (WJS) in 1985 at the University of Florida horticultural farm, six miles NW of Gainesville. Another (FLAS F 55643) was collected in 1990 by another of the authors (BPA) in Hague, 10 miles N of Gainesville. The other four Florida collections (FLAS F 9656, F 9697, F 18056, and F 19496) date from 1927 to 1938, and came from various sites in and around Gainesville. Site locations within Alachua county are presented in Fig. 1. Florida collections were chosen from different sites in Alachua County to ensure that different genets were sampled, thus allowing for variation at an individual level to yield a set of data reflecting characteristics of the population as a whole. Collections were selected from a population within a single county, i.e., a region small enough that hyphae from one might reasonably be able to mate with those from any of the others. The Illinois population was represented by the single collection available for study. Multiple single-population collections from Florida were utilized to generate a well rounded set of data on the population.

Another potential confounding factor considered was the likelihood that within a single collection, longer spores might tend to be proportionately narrower or broader on average than shorter spores. If this were the case, analytic error could result from spores of one data set having length values different from those of the other (Pedhazur, 1982). To control for this possibility, spore lengths were carefully equilibrated. For each spore measured from Illinois, one of equal length was selected from the Florida collections. This served to enhance the statistical validity of the comparison between the two populations. A total of 30 spores, 15 from each population, constituted the sample size.

This study thus involved the use of two predictor variables, one continuous and the other categorical with two levels, including study of intervariable interaction. The level of significance (α) was set at 0.05, establishing a 95% degree of confidence for the results. The hypothesis proposed that regression of spore width on length would vary between the two populations.

RESULTS

Spore width in relation to length differed significantly between the two populations. Collections from Florida displayed a mean width of 6.4533 μm, a range of 5.5 to 7.7 μm, a standard deviation of 0.5927, and a 0.68193 correlation of length with width. The Illinois collection displayed a mean width of 7.0867 μm, a range of 6.5 to 8.0 μm, a standard deviation of 0.4704, and a 0.70552 correlation of length with width.

Full model correlation analysis indicated high overall significance ($p < 0.0001$), with length as the only significant single predictor ($p < 0.0001$). When the effect coded interactive term was removed, however, locale also became a significant predictor ($p < 0.0002$). These results are summarized below in Tables 1 and 2.

Analysis was also conducted to obtain coefficients of regression. The overall equation obtained for the full model was:

$$Y' = 3.022 + 0.384(\text{Length}) - 0.685(E) + 0.038(E*\text{Length})$$

This model yielded separate regression equations for each locale:

Florida: $Y' = 2.337 + 0.422(\text{Length})$
Illinois: $Y' = 3.707 + 0.346(\text{Length})$

Because of the nonsignificance of the interaction between the two predictors, regression analysis testing only the main effects was conducted. The overall regression equation resulting from this revised model was:

$$Y' = 3.022 + 0.384(\text{Length}) - 0.317(E)$$

This generated revised regression equations for each of the two populations:

Florida: $Y' = 2.706 + 0.384(\text{Length})$
Illinois: $Y' = 3.339 + 0.384(\text{Length})$

To determine the best predictive model, each of the two X variables by itself was treated to regression analysis. A simple analysis using only length indicated significance ($p <$

0.0007), which was slightly diminished from the two-predictor model. The associated regression equation obtained was:

$$Y' = 3.022 + 0.384(\text{Length})$$

Tested by itself, locale was still significant ($p < 0.0031$), but again to a diminished degree. The regression equation resulting from this model was:

$$Y' = 6.77 - 0.317(E)$$

For the two locales, separate regression equations were obtained:

Florida (Locale 1): $Y' = 6.453$
Illinois (Locale 2): $Y' = 7.087$

These two predicted values correspond exactly to those obtained for average width by correlation analysis. Analysis thus showed that the best predictive model was the one incorporating main effects only. The regression equations for the separate locales from that model were used to calculate predicted width values (Y') for minimum and maximum spore lengths of 8.6 and 12.7 μm, respectively. The values this procedure generated were 6.0084 and 7.5828 for Florida; and 6.6414 and 8.2158 for Illinois. These data points were plotted to scale and their corresponding regression lines drawn (Fig. 2).

DISCUSSION

Considering the climatic difference between Illinois and Florida, the tendency toward a lower length/width spore ratio in the more northern population of *Leucoagaricus hortensis* is interesting. One question it raises is whether other basidiomycete species are typified by such variation in spore ratio between geographically separated populations. If so, a further question would arise of whether this variation is random, such that a northern population of some other species might have spores which are narrower on average than those from a southern population. This would be the reverse of the case observed here for *L. hortensis*. Equally compelling, however, is the possibility of patterning in such variation, with northern populations in general tending to exhibit spores that are proportionately broader, per unit length, than southern ones. If this were the case, an explanation for such patterning might be found in the climatic differences between northern and southern latitudes. For example, greater width in relation to length might confer some greater degree of resistance to desiccation and cold in a climate with comparatively harsh winters. This possible adaptive significance could thus reflect an environmental selection factor. Further investigation will be necessary to test this hypothesis.

CONCLUSION

Length and locale are both significant predictors of basidiospore width in *Leucoagaricus hortensis*. Length is the better single predictor, but the best model uses both variables, and is able to account for 62% of the variance in spore width. Based on this model, the odds are less than 2 in 10,000 that the width-per-unit-length variation observed here between Illinois spores and Florida spores would occur by random chance. Analysis thus supported the hypothesis that there would be a significant difference between populations in regard to spore ratio. This difference is considered here as an allopatric variation, because of its statistically significant association with populations in different geographic

regions. An interesting future direction for research is the question of whether *L. hortensis* is unique in this regard or whether other species of basidiomycetes display similar variation between northern and southern populations. In the meantime, a cautionary note is sounded regarding the use of average basidiospore ratio in taxonomic identification of agarics, because of demonstrated variability of this character between populations of the species studied here.

ACKNOWLEDGMENTS

The authors would like to thank James Kimbrough, curator of the fungal collections of the University of Florida herbarium (FLAS), for loaning specimens of *Leucoagaricus hortensis* for study. Also, David Gibson, Keith Fessel and Patricia Woelkhe are thanked for helpful comments offered about this research.

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Figure 1. Florida collection sites of *Leucoagaricus hortensis*.

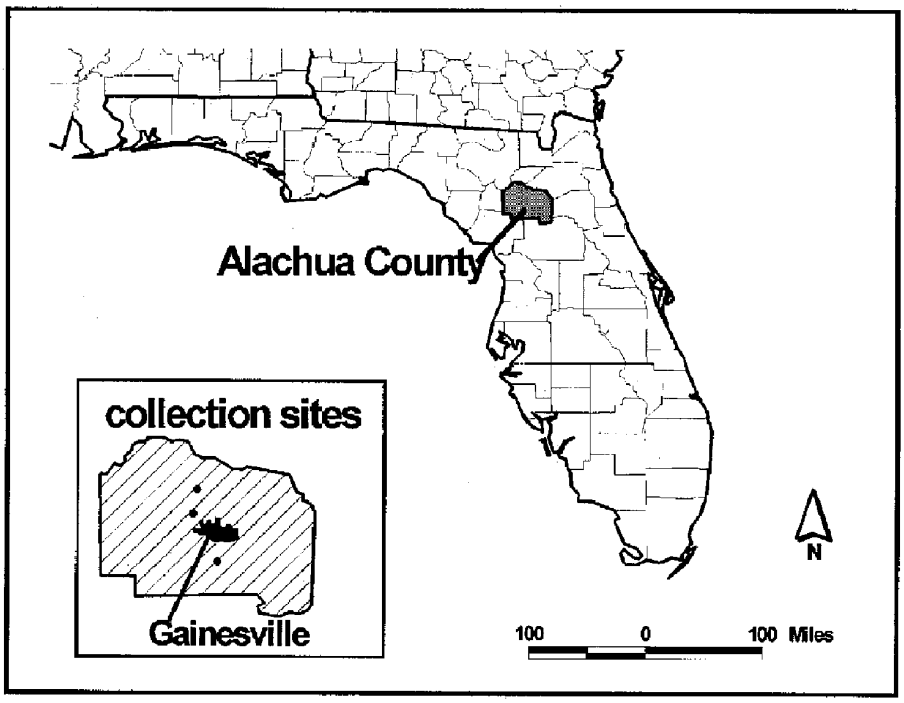

Table 1. Summary table for full model using Proc GLM (SAS 1989).

Dependent variable = Spore width

SOURCE	df	SS	MS	F-value	p-value
Regression	3	6.837	2.279	14.15	<0.0001
Residual	26	4.186	0.161		
Total	29	11.023			

R-squared = 0.62

SOURCE	df	Type III SS	F-value	p-value
Length	1	3.792	23.55	<0.0001
Locale	1	0.125	0.78	<0.3854
Length*Locale	1	0.037	0.23	<0.6375

Table 2. Summary table for revised model using Proc GLM (SAS 1989).

Dependent variable = Spore width

SOURCE	df	SS	MS	F-value	p-value
Regression	2	6.800	3.4	21.74	<0.0001
Residual	27	4.222	0.156		
Total	29	11.023			

R-squared = 0.62

SOURCE	df	Type III SS	F-value	p-value
Length	1	3.792	24.24	<0.0001
Locale	1	3.008	19.23	<0.0002

Figure. 2. Regression lines of spore width x length for two populations of *Leucoagaricus hortensis* (*represents two data points).