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HERITABLE VARIATION AND MECHANISMS OF INHERITANCE OF SPORE SHAPE WITHIN A POPULATION OF *SCUTELLOSPORA PELLUCIDA*, AN ARBUSCULAR MYCORRHIZAL FUNGUS[1]

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Substantial variation was found among single-spore cultures established from a single population of the arbuscular mycorrhizal fungus *Scutellospora pellucida*. A common environment experiment demonstrated that five single-spore cultures differed in their average spore shape (as measured by length:width ratios) and size (volume) with interisolate heritabilities of offspring mean values of 0.96 and 0.87, respectively (0.66 and 0.43 for the shape and size of individual spores). The distribution of offspring spore shapes also differed in levels of variance, skewness, and kurtosis. In fact, these aspects of the distributions shifted with mean spore shape as predicted by the binomial distribution—the distribution expected due to the segregation of genetically diverse nuclei through dividing hyphae. Thus, the original parental spores generating these cultures appear to have contained genetically variable nuclei, which then segregate into the offspring spores to generate consistent differences in the mean, variance, skewness, and kurtosis of the distribution of offspring spore shapes. This nuclear segregation may be followed by the assemblage of novel combinations of nuclei through hyphal fusion. Together these processes are rarely considered mechanisms for the creation of novel genetic combinations and may contribute to the maintenance of the high level of heritable variation observed in this study.

Key words: arbuscular mycorrhizal fungi; asexuality; genetic variation; glomalean fungi; heritabilities; heterokaryon; spore morphology.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts associating with an estimated 80% of all plants including the majority of food crops (Trappe, 1987). Currently AM fungi are thought to be predominantly asexual, though sexual zygospores have once been reported for an isolate of *Gigaspora decipiens* (Tommerup and Sivasithamparam, 1990). In the absence of the variation-generating activity of sexual reproduction, the variation-reducing effects of directional selection and drift may be expected to pauperize populations of genetic variation, although substantial genetic variation has been found within populations of other putatively asexual fungal species (e.g., McDonald et al., 1995; Geiser, Pitt, and Taylor, 1998). AM fungi, however, are multinucleate, with the number of nuclei in individual spores ranging from 2600 to 3850 in *Gigaspora gigantea* and *Scutellospora erythropa* (Cooke, Gemma, and Koske, 1987; Bécard and Pfeffer, 1993), and it is possible that nuclei within a single-spore are genetically variable (Sanders et al., 1995; Lloyd-MacGilp et al., 1996; Sanders, Clapp, and Wiemken, 1996). In such a case, a process analogous to sexual reproduction may work to generate heritable phenotypic variation (Sanders, Clapp, and Wiemken, 1996). First, genetically diverse nuclei may segregate into diverging hyphae (analogous to independent assortment of genetically distinct chromosomes into gametes during meiosis). A second process, that of fusion of genetically distinct hyphae, may then create novel combinations of nuclei (analogous to the sexual recombination of chromosomes). The potential of such a genetic mechanism to create and maintain heritable variation is unknown.

Previous studies have found heritable phenotypic variation between populations of the same AM fungal species (Stahl, Christensen, and Williams, 1990; Sylvia et al., 1993). Geographically separated populations have been found to vary in ecologically important traits, including ability to increase tolerance of their host to heavy metals (Gilden and Tinker, 1981; Weissenhorn, Leyval, and Berthelin, 1993), tolerance of their host to drought (Stahl and Christensen, 1991; Bethlenfalvay et al., 1989), and phosphorus uptake of their host (Louis and Lim, 1988). The ecotypic variation observed in these studies suggests that AM fungi are capable of adapting to their local environmental conditions.

The process of adaptation requires the existence of heritable phenotypic variation within a population. However, up to now there is little published evidence of intrapopulation heritable phenotypic variation within AM fungi. Recent molecular studies demonstrated the existence of genetic variation in molecular markers within populations of these fungi (Sanders et al., 1995; Dodd et al., 1996; Lloyd-MacGilp et al., 1996; Sanders, Clapp, and Wiemken, 1996). The investigation of inheritance of phenotypic variation is limited in these fungi by the availability of easily scored morphotypes. In a previous study, we used a selection experiment to document the presence

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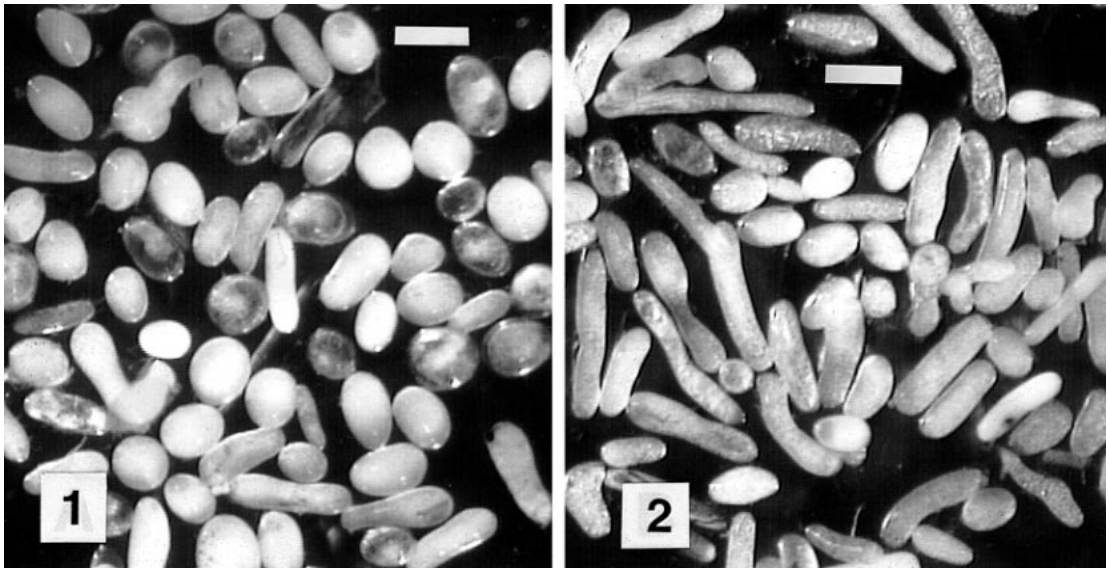


Figs. 1–2. A random sampling of progeny spores derived from two single-spore cultures of *Scutellospora pellucida.* Both isolates *S. p.* B and *S. p.* C, shown in Figs. 1 and 2, respectively, have round and oblong spores. However, spores of *S. p.* C are, on average, more oblong and smaller than those of *S. p.* B. Scale bar = 250 μm.

of heritable quantitative variation in spore size and color within a laboratory isolate of *Glomus clarum* (Bentivenga, Bever, and Morton, 1997).

In this study, we investigated the existence and inheritance of genetic variation in spore shape within a single natural population of *Scutellospora pellucida* [Nicol. & Schenck] Walker & Sanders. *Scutellospora pellucida* was originally described as having globose to subglobose spores (Koske and Walker, 1986); however, we noticed that many spores of *S. pellucida* at our study site were highly elongated, sometimes with lengths of 1 mm or greater (Figs. 1, 2). We evaluate the genetic basis for this variation by deriving cultures from single spores and then characterizing them by growing them in a common environment. Measurement of growth within a common environment is a standard technique to determine what component of total phenotypic variation is heritable (Turesson, 1922; Falconer, 1989). In the absence of a heritable component, the isolates would produce spores of similar size and shape when grown in a common environment. Besides mean spore shape and size, we also analyzed the variance, skewness, and kurtosis of the distribution of spore characters. These aspects of the distribution provide insight into the genetics of AM fungal populations and the processes of genetic segregation.

MATERIALS AND METHODS

All fungi were collected from a 5 × 15 m area of a well-studied mown grassland as part of an investigation into the AM fungal host-dependent growth rates and species diversity (as described in Bever et al., 1996). Trap cultures were started in September 1992 by filling 6 × 25 cm pots with a mixture of fresh field soil and sterile sand and planting into the pot mycorrhizae-free plants of *Allium vineale* L., *Anthoxanthum odoratum* L., *Panicum sphaerocarpon* Ell., *Plantago lanceolata* L., or *Sorghum vulgare* Pers. (Bever et al., 1996). These cultures were then grown in a greenhouse in North Carolina under natural sunlight and cool conditions (4°–21°C), watered as needed, and fertilized during the 2nd and 4th mo with 100 mL of one-quarter strength Hoagland's solution modified to contain a reduced concentration of phosphorus (Millner and Kitt, 1992). The pots were harvested after 4.5–6 mo and stored in a sealed plastic bag at 4°C for up to 1 mo until spores could be extracted (Bever et al., 1996). Spores were extracted from the soil by blending ~100 mL of soil in water for two 5-s bursts in a Waring blender, collecting the spores by pouring the slurry through a 45-μm mesh sieve, and separating the spores from sand, soil, and organic debris using sucrose gradient centrifugation (Daniels and Skipper, 1982). Spores were identified as *S. pellucida* on the basis of size, color (white to yellow with a tan germination shield), smooth surface, and wall structure (Franke and Morton, 1994).

Healthy spores of *S. pellucida* were selected on the basis of a clean smooth surface and organized, clear fat globules in the spore lumen. Single-species cultures were established according to the procedures of Morton, Bentivenga, and Wheeler (1993) and are maintained as NC155 in the International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM) (Morton, Bentivenga, and Wheeler, 1993). Single-spore cultures were started by placing single spores on the roots of 10–12 d-old *Sorghum sudanense* [Piper] Staph plants grown in steamed soil. Plants were then transferred to a steamed mixture of sand and soil (1:1) from the NC field site in 3 × 20 cm pots. A total of 25 cultures were initiated and grown in a heated greenhouse in West Virginia with supplemental light. Cultures were harvested and sampled after 4–5 mo. Five cultures successfully produced spores. Spores for these successful isolates, which we refer to as *S. p.* A–*S. p.* E, were originally isolated from trap cultures with *Plantago* (*S. p.* A and B), *Panicum* (*S. p.* C and E), and *Allium* (*S. p.* D).

To test whether observed differences in the shape and sizes of spores from these five isolates were due to heritable differences between the isolates, we grew four replicates of each isolate in randomized order within the same environment. These second generation or ''offspring'' cultures were started from ~20 mL of soil of the parent cultures mixed into the steamed field soil-sand in 3 × 20 cm pots. *S. sudanense* was grown in each pot 5 mo in a greenhouse as described above. All 20 cultures produced spores.

For cultures of both parent and offspring generations, spores were extracted from ~50 cm^3 of soil. Mature spores were identified by their darkly pigmented germination shield (Franke and Morton, 1994). Up to 56 mature spores per pot were measured in the order that they were encountered under the stereoscope. The length (measured along spores'

longest axis) and width (measured at widest point perpendicular to the spores' longest axis) of each spore were measured using an optical micrometer under a binocular stereomicroscope. Color of spores in the offspring generation was estimated by comparison with an INVAM color chart (available from the authors) simultaneously under fiber optic illumination at 3400°K.

Statistical analysis and interpretation—Measures of spore length (l) and width (w) were converted into measures of spore shape (oblongness) and size (volume). Spore shape was characterized by the ratio of spore length over spore width (l/w). Spore volume was calculated using the measures of spore length and width and the equation for volume of a prolate spheroid ($V = [lw^2]/6$). Analyses of variance were performed on descriptors of the distributions of spore shape and size for each replicate, including the mean, variance, coefficient of variation (the ratio of the variance over the mean), skewness, and kurtosis. Because the number of spores measured varied between pots (with the fewest in a pot being 24 spores) and these differences in sample size could lead to biases in the estimates of variance (including the coefficient of variation), skewness and kurtosis, all analyses were performed on the average of 1000 jackknifed estimates of each of these descriptors (Sokal and Rohlf, 1995). Specifically, for each replicate pot, 1000 estimates of each descriptor were calculated from the measurements of 24 spores, which were randomly chosen without replacement from the raw data. The color estimates were ranked by darkness, and analyses were performed on these ranks.

Because replicates of isolates experienced variation in the environment at random (i.e., because of the randomized design), consistent differences between the isolates can be attributed to heritable (in the broad sense) differences between the isolates. In the absence of heritable differences between the isolates, no significant isolate effect would be detected. Therefore, the existence of heritable variation among the isolates was analyzed using an analysis of variance in the offspring generation using the general linear models procedure of SAS (SAS, 1986). Isolates were treated as random effects. When these tests were significant, means of the isolates were compared while controlling for multiple comparisons using Tukey's minimum significant difference criterion (SAS, 1986). Variance components were estimated from the analysis of variance, and interisolate heritabilities were calculated as the ratio of interisolate variance (V_i) over the total variance ($V_i + V_e$, the environmental variance). As these analyses are done on descriptors of the offspring spore distributions (e.g., the averages), the interisolate heritabilities do not reflect the heritabilities of the shape and size of individual spores. The interisolate heritabilities for the shape and size of individual spores were calculated by adjusting the phenotypic variance components by the average variation in these measures observed among spores from single pots.

Inference into the mechanism of inheritance of spore shape—The observed distributions of spore shape were compared to those expected due to the segregation of genetically variable nuclei into the offspring spores. If we imagine that spore shape is determined by the composition of nuclei in the hyphae and that there are two nuclei types, with one encoding for round spores and the second encoding for oblong spores, then the segregation of these nuclei from an initially heterokaryotic parental spore would produce both round and oblong spores (as depicted in Fig. 3a). In fact, the random segregation of parental nuclei into progeny spores would generate a distribution of offspring spore types as described by the binomial distribution. As detailed in the Appendix, these distributions would be functions of the frequency of nuclei types in the parental spore (p), the number of parental nuclei that contribute to the phenotype of an individual offspring spore (n), and the translation of these nuclei into phenotypic changes of spore shape (a). We obtained estimates of n and a by regressing the ratio of the observed variance over the observed mean spore shape against the mean spore shape using Eq. 4. derived in the Appendix. We then calculated the parental frequencies of each isolate (p_i) using the observed mean spore shape. These estimated values were used to generate the expected binomial distributions. To evaluate the ability of the binomial distribution to predict the observed shifts of the variance, skewness, and kurtosis with the mean, we regressed the observed variance, skewness, and kurtosis against those of the expected distributions.

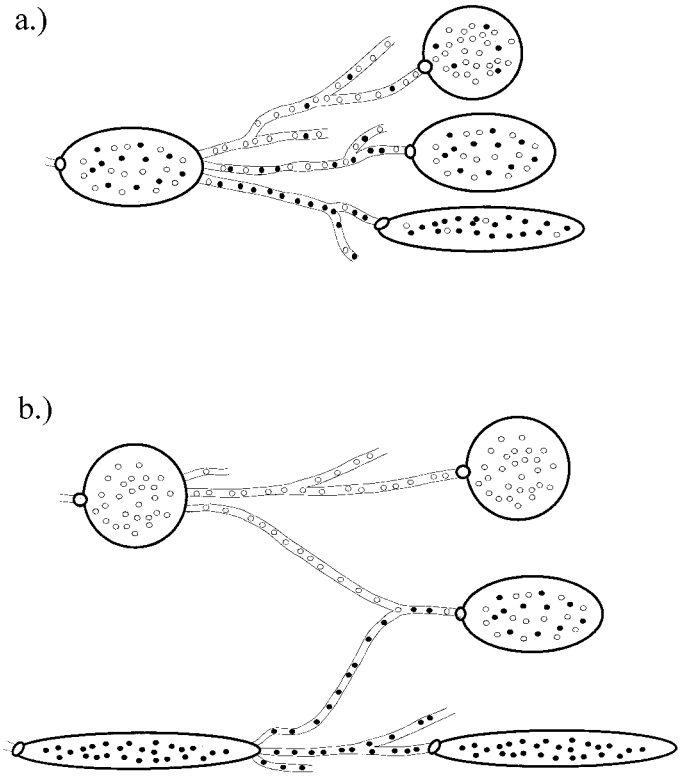


Fig. 3. Proposed mechanism of inheritance of spore shape in *S. pellucida.* The parental spores are depicted on the left and the offspring spores are depicted on the right. In (a), the segregation of nuclei that code for round spores (white) and oblong spores (black) from a single ovoid spore into round, ovoid, and oblong spore types is shown. In (b), hyphae from genetically distinct round and oblong spores merge to produce an ovoid spore.

RESULTS

Substantial heritable variation existed for the average size and shape of spores (Table 1). In fact, a posteriori comparisons of means demonstrate that four of the five isolates were distinct, with all isolates except *S. p.* D and *S. p.* E being statistically distinguishable (Fig. 4). However, no evidence of interisolate variation in mean spore color was observed. While the estimates of interisolate heritabilities for means of spore size and shape were very high (Table 1), after adjusting for the within-pot variation, we estimate the interisolate heritabilities of the shape and size of individual spores to be 0.66 and 0.43, respectively.

The single-spore isolates also differed significantly in the variance, skewness, and kurtosis of spore shape and size (Table 1, Fig. 5). The variance in spore length : width ratio increased with the average length : width ratio of the spores (Fig. 5). Such an allometric increase in variance is expected for measures of size. However, while this

TABLE 1. Analysis of variance table and estimates of interisolate heritabilities for descriptors of the distributions of spore shape and size from five single-spore isolates. Individual measures were made in four replicate pots from each isolate. The interisolate heritabilities were calculated as the ratio of the variance component due to the single-spore isolate over the sum of this interisolate variance component and that due to variance in the environment.

Response	Sum of squares isolate (df = 4)	Sum of squares error (df = 19)	Significance of isolate effect ($P <$ value)	Interisolate heritabilities
Mean length:width ratio	18.56	0.85	0.0001	0.95
Mean volume	3.91×10^{14}	5.24×10^{13}	0.0001	0.87
Variance length:width ratio	40.52	4.18	0.0001	0.90
Variance volume	1.08×10^{28}	8.6×10^{27}	0.01	0.48
CV length:width ratio[a]	1344	670	0.0016	0.62
CV volume[a]	360	385	0.03	0.38
Skewness length:width ratio	2.77	2.02	0.008	0.51
Skewness volume	1.16	5.15	ns	ns
Kurtosis length:width ratio	62.76	76.3	0.05	0.34
Kurtosis volume	4.9	31	ns	ns

[a] CV represents the coefficient of variation.

allometric effect could explain much of the differences in the variance, the isolates also differed in their coefficients of variation of length : width ratio (Table 1), demonstrating significant heterogeneity of variance between isolates beyond that expected by simple allometry. The isolates differed in the skewness of the distributions of spore shape (Table 1), with the right skewness of the distribution of spore length : width ratio in *S. p.* A decreasing with the mean (Fig. 5). The kurtosis of the distributions of spore shape also differed between the isolates, with isolate *S. p.* E having the most platykurtic distribution while *S. p.* A had the most leptokurtic distribution.

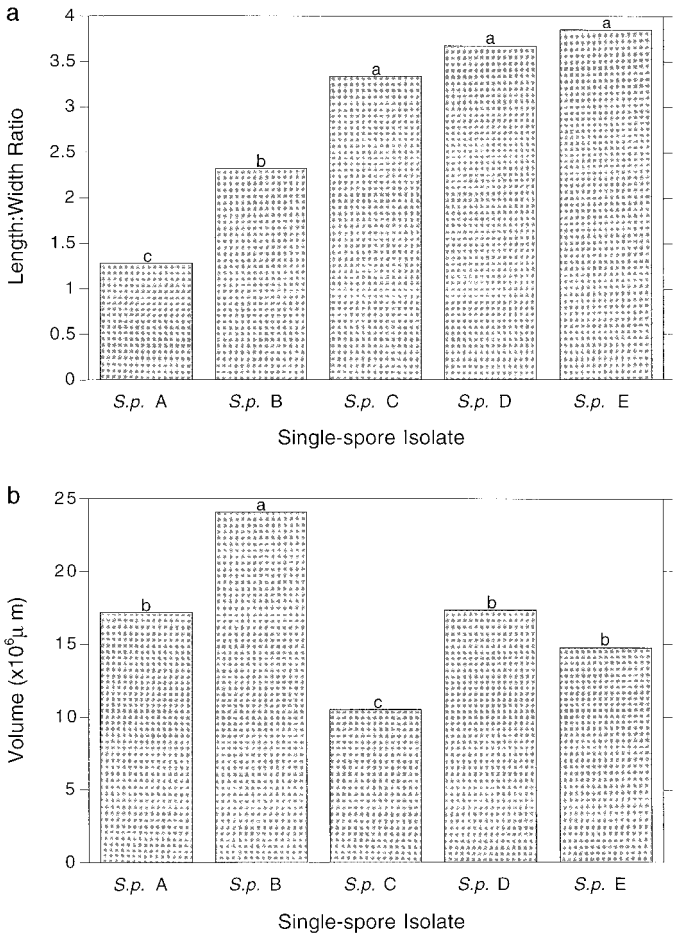
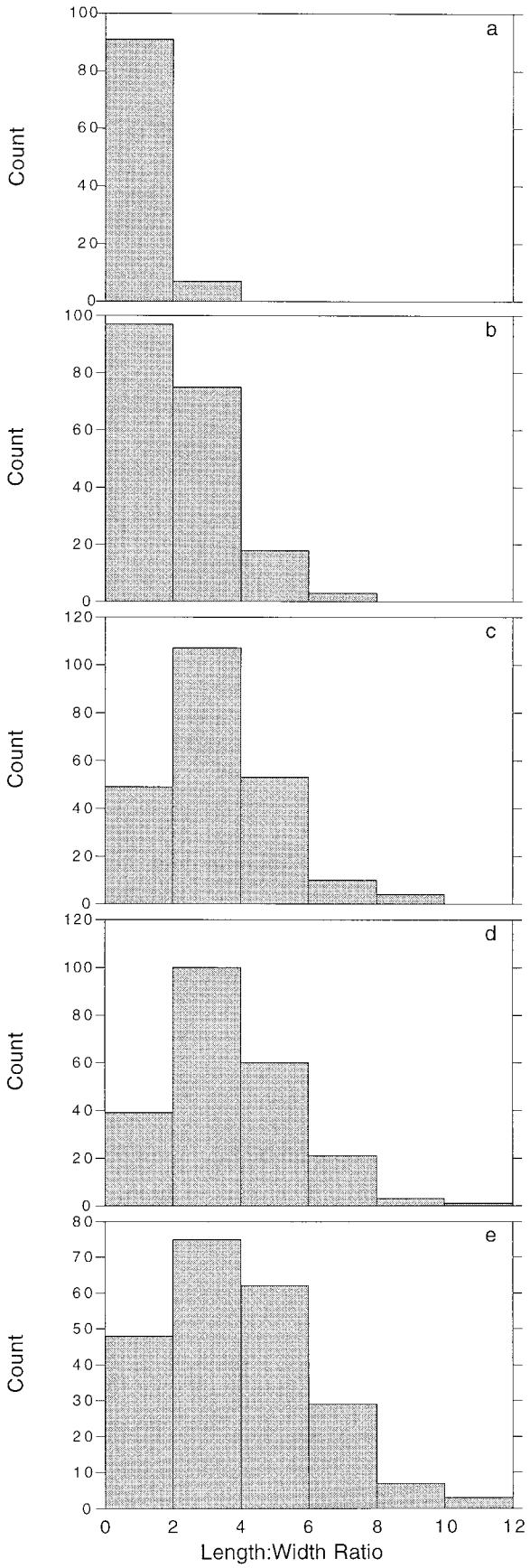


Fig. 4. The five single-spore isolates of *S. pellucida* differed in their average spore shape (length : width ratio) and volume, as depicted in (a) and (b), respectively. Different lowercase letters indicate significant differences at $P < 0.05$ as determined using Tukey's minimum significant difference.

Inference into the mechanism of inheritance of spore shape—Using the regression of the observed variance: mean ratios against the means (Eq. 4 derived in the Appendix), we estimated the number of parental nuclei that contribute to an individual progeny spore (n) to be 7 and the contribution of each oblong nuclei to the length : width ratio (a) to be 1.65. This regression was not significant and was dependent upon the specified value of b, so we do not place great confidence in these particular values. Nevertheless, these estimates do allow us to demonstrate that the shift in the variance, skewness, and kurtosis with the mean is consistent with that expected by a binomial distribution. By calibrating the parental frequencies of nuclei types (p_i) with the observed mean of each isolate, we obtain idealized binomial distributions for the five isolates (Fig. 6) which are very similar to the observed distributions (Fig. 5). In fact, the values of variance, skewness, and kurtosis expected by the binomial distribution provide good predictions of the observed values, with significance levels of 0.006, 0.002, and 0.002, respectively (Table 2). The significant relationship between observed and expected variance might be expected given that the observed variance was used to estimate the parameters of the binomial distribution. The high significance of the regressions of skewness and kurtosis, however, is particularly notable, since these values were not used in the derivation of n and a.

DISCUSSION

This study documents that heritable phenotypic variation can persist within a single natural population of an asexual arbuscular mycorrhizal fungus. The high level of heritable variation observed is particularly remarkable given our small sample size. Of the five single-spore isolates compared, four of them had distinct morphological



distributions (Fig. 4). These consistent differences between the isolates accounted for 96 and 87% of the total variation in the average shape (length/width) and size (volume), respectively, of spores in a pot (Table 1). We note, however, that because of the high level of variation within pots, the estimates of interisolate heritabilities for the shape and size of individual spores are substantially reduced (0.66 and 0.43, respectively). Nevertheless, these heritabilities remain considerably larger than the heritabilities realized in a selection experiment using a laboratory culture of the arbuscular mycorrhizal fungus *Glomus clarum* (Bentivenga, Bever, and Morton, 1997).

This study further presents the first documentation of significant differences in the levels of variation produced by individual spores. These differences are not a simple correlate of differences in average spore size, as coefficients of variation are also significantly different. Moreover, the offspring-generation distributions of spore shape also differed in their skewness and kurtosis. These shifts provide important clues as to the mechanism of inheritance of spore characters in these fungi (discussed below).

The heritable variation within this population could be passively maintained through a balance of mutation and genetic drift or actively maintained by balancing selection. Given the multinucleate nature of the spores and hyphae, the strength of genetic drift would depend upon the effective size and the effective generation time of the population of nuclei. These measures will depend upon the life history, density of spores and hyphae, segregation patterns of the nuclei in branching hyphae, and the rates of hyphal branching and hyphal fusion, all of which are poorly understood.

The high heritability of the mean values of spore size and shape suggests that these characters could evolve in response to selection. In fact, we have observed a phenotypic response to a single generation of selection on spore size within another species of AM fungi (Bentivenga, Bever, and Morton, 1997). While spore shape does not have any obvious selective value, spore volume, a reproductive character, is likely to be under strong selection. In fact, spore volume is less variable than might be expected given the variation in length and width. However, the intensity of selection on spore volume may be offset by a trade-off in spore number as has been found for size and number of reproductive propagules in other organisms (e.g., in goldenrods: Werner and Platt, 1976).

The developmental mechanism by which the divergent spore shapes are produced is unknown. From previous work on the developmental sequence within an isolate of *S. pellucida* (Franke and Morton, 1994), we know that maturation of spores is related to the addition of spore walls and germination shields, which are independent of the shape of the spore itself. Cursory observations of ex-

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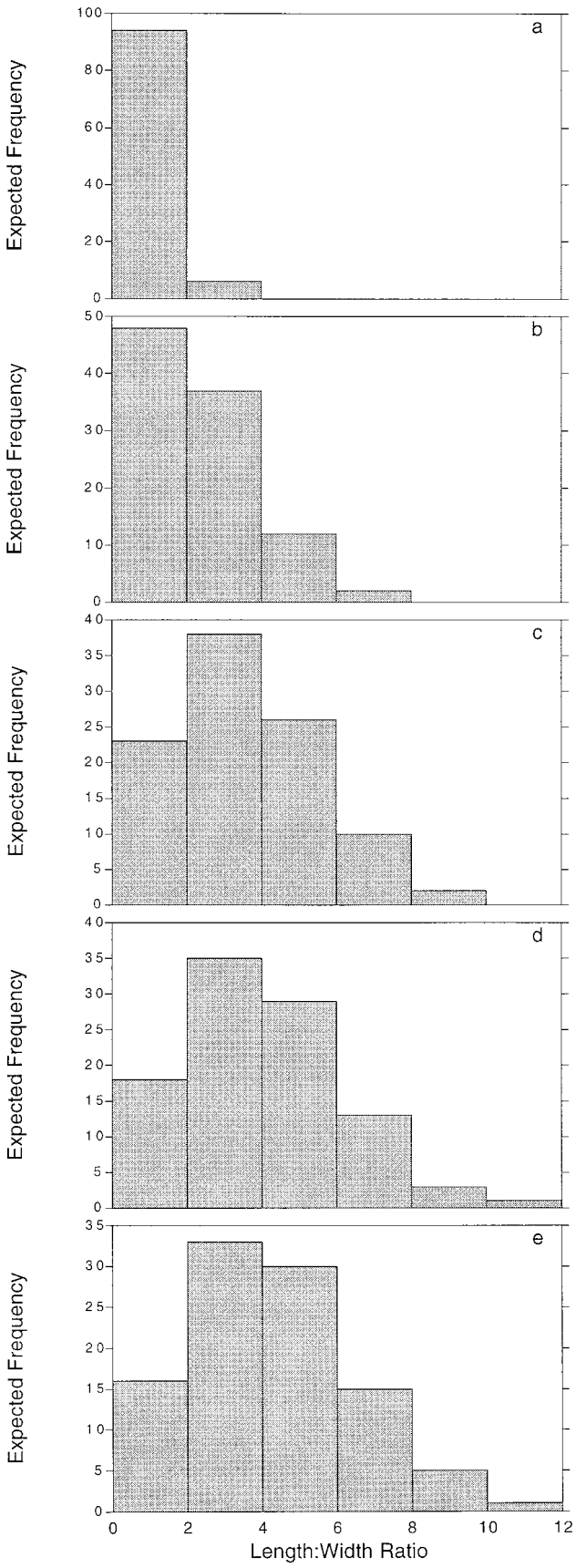
Fig. 5. Distributions of spore shape (length : width ratio) of five single-spore isolates of *S. pellucida.* The mean, variance, skewness, and kurtosis of the distributions differ among isolates *S. p.* A through *S. p.* E, as depicted in (a) through (e). For example, the distribution of *S. p.* A has a substantially reduced variance, but a greater skewness and kurtosis than that of the other isolates.



tracted spores suggested that shape may be a by-product of the location in which the spore begins development, with elongated morphologies being frequently observed within the cortex of plant roots (even plant roots, which are not much wider than the spore). However, the spherical spores of isolate *S. p.* 1 were found in situ to have been initiated within the cortical region of roots and subsequently to have emerged from the root by splitting the cortex (James D. Bever, personal observation), suggesting that the divergent morphologies are not simply due to site of initiation of spore development. Regardless of the developmental mechanism, spore shape is a heritable, tractable trait, which may provide much-needed insight into the nature of the genetic system in AM fungi.

Mechanism of inheritance of spore shape—We identify two alternative mechanisms of inheritance of spore shape in these asexual fungi, given that the genes that encode for spore shape are located on chromosomes within nuclei: either the parental spores contain genetically identical nuclei with respect to spore shape (i.e., they are homokaryotic) or the parental spores contain genetically different nuclei with respect to spore shape (i.e., they are heterokaryotic). The homokaryotic scenario cannot easily explain our observed results. To explain the observed differences in average spore shape under the homokaryotic scenario, for example, would require that there be at least four distinct nuclei types. However, in this homokaryotic spore scenario, we would also have to posit that these nuclei also differed in genes that generate various levels of variance, skewness, and kurtosis of spore shape. A mechanism generating these differences in offspring spore shape distribution is difficult to imagine.

Alternatively, the heritable differences in mean shape, as well as heritable differences in the variance, skewness, and kurtosis could result from the original spores being heterokaryotic, with the five isolates varying in their proportions of as few as two types of nuclei. If we imagine that nuclear ratios determines the phenotype of AM fungi, as has been observed in other heterokaryotic fungi (Burnett, 1975), then the segregation of two nuclei types (as depicted in Fig. 3a) could generate the observed shifts in the mean, variance, skewness, and kurtosis of offspring spore shape. The segregation of two nuclei types from one generation to the next would be expected to generate offspring spore shapes as characterized by binomial distributions. Thus, spores with a predominance of nuclei with genes for round spores would be expected to produce a majority of round spores and a few narrow spores resulting in a low mean, variance, and kurtosis of the length : width ratio, but a high skewness (e.g., *S. p.* A, Figs. 5a and 6a). Alternatively, spores containing a higher proportion of nuclei with genes for oblong spores produce an offspring generation of spores with a higher mean, variance, and kurtosis of their offspring spore

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Fig. 6. Distributions of spore shape predicted by the binomial distribution. The distributions predicted for *S. p.* A through *S. p.* E are presented in (a) through (e), respectively. The parameters for the predicted distributions were derived using the observed mean and variance and then calibrated to the mean of each isolate as described in the Appendix.

TABLE 2. Regression of average values of variance, skewness and kurtosis of spore shape of five isolates against the corresponding values predicted by the binomial distribution. The binomial distributions were calibrated using the average spore shapes for the five isolates as described in the Appendix.

Average observed values	R^2 (model df = 1) (error df = 3)	Significance ($P <$ value)
Variance length:width ratio	0.941	0.006
Skewness length:width ratio	0.974	0.002
Kurtosis length:width ratio	0.971	0.002

length : width ratio, but a less skewed distribution (e.g., *S. p.* E, Figs. 5e and 6e). Our data support this pattern with our observed distributions (Fig. 5) closely matching the distributions of the idealized binomial distribution (Fig. 6), and the observed shifts in variance, skewness, and kurtosis of the distributions are very well predicted by the idealized distribution (Table 2). We can then suggest that the five parental spores that generated these isolates were heterokaryotic and contained unique populations of genetically variable nuclei. The possibility that spores of AM fungi are heterokaryotic is supported by the observation of genetically divergent sequences of the ITS region of ribosomal RNA within single spores of *Glomus mosseae*, *G. dimorphicum*, and *G. coronatum* (Sanders et al., 1995; Lloyd-MacGilp et al., 1996; Sanders, Clapp, and Wiemken, 1996).

Our results, then, support the operation of a rarely considered mechanism in Glomales for creating novel genotypic combinations (Sanders, Clapp, and Wiemken, 1996) where genetically diverse nuclei segregate through dividing hyphae to generate genetic variation between progeny spores (Fig. 3a). The possible existence of such a mechanism in other fungi had previously been identified, though not confirmed (e.g., Burnett, 1975). Over time, this process would segregate variation within a hypha into variation between hyphae in a process analogous to selfing in sexual populations. Nuclear variation existing between hyphae could then be recombined through hyphal fusion (illustrated in Fig. 3b), to reconstitute genetically diverse hyphae in an analogous fashion as outcrossing in sexual populations. While hyphal fusion remains an undocumented component of this mechanism for AM fungi, fusion of genetically distinct hyphae is known in other fungi, though it is thought to be uncommon in the Mucorales, the best studied group with Zygomycetes (Burnett, 1975; Alexopoulos and Mims, 1979). Our results indirectly support the operation of fusion of genetically distinct hyphae in *S. pellucida*, since nuclear segregation alone over many generations would otherwise render individual spores homokaryotic—which, as we have found, they are not.

Together, the joint action of hyphal segregation of nuclei and the subsequent fusion of genetically distinct hyphae may contribute to the maintenance of substantial genetic variation, even in the absence of sexual recombination. While the possibility of infrequent sexual or parasexual recombination of chromosomes cannot be eliminated within this apparently asexual fungus, we have found support for an alternative mechanism that could contribute to the maintenance of the high level of heritable variation, which we observed within a single population of *S. pellucida.*

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APPENDIX

Derivation of expected distributions due to segregation of two nuclei types.

If we imagine two nuclear types within the original parental spore that determine spore shape (as illustrated in Fig. 3a), the random segregation of genetically diverse nuclei through hyphal division would generate a binomial distribution characterized by the initial frequency of the two nuclear types (p) and the effective number (n) of these parental nuclei, which segregate into and contribute to the phenotype of each offspring spore. This latter value (n) will itself be a function of many poorly understood aspects of the biology of *S. pellucida*, including the rate of hyphal elongation and division and the rate of nuclear division. For our purposes, we consider these biological processes and the effective number of parental nuclei (n) as constant across all isolates. We are then interested in knowing whether the observed shift in the variance, skewness, and kurtosis of the distribution of spore shape with mean spore shape was consistent with that generated by the idealized binomial distribution in which the initial frequency of two nuclei, p, was set by the observed mean.

We then want to fit binomial distributions to the observed distributions (as depicted in Fig. 5), while holding n constant and varying p. To derive the value for n, we hypothesize that spore shape is a linear function of the number of nuclei encoding for oblong genes, which contribute to the development of that spore, as follows:

$$S = aL + b, \tag{1}$$

where S represents the spore length:width ratio, L the number of nuclei encoding for the oblong spore shape that contribute to that spore's development, and a and b are constants. Provided n nuclei are chosen at random with replacement from the parent spore, the mean spore shape generated by that parent will then be

$$\text{Av}[S] = anp + b, \tag{2}$$

where p represents the frequency of nuclei encoding for oblong spore shape in the parent spore. The variance, as given in standard probability texts (e.g., Ross, 1984), will be

$$\text{Var}[S] = a^2np\ (1 - p). \tag{3}$$

By rearranging Eq. 3 and substituting in Eq. 2, we derive

$$\text{Var}[S]/(\text{Av}[S] - b) = a - (1/n)(\text{Av}[S] - b). \tag{4}$$

Therefore, by regressing the observed variance/mean ratio against the mean as in Eq. 4, we can estimate the value a as the x intercept and the value m as the negative of the inverse of the slope, assuming that we know the value of the constant b. For length : width ratio the value of b must be at least 1, as that is the minimum ratio. That is, even a spore with no oblong nuclei will have a value of S of at least 1. This value will actually be greater than 1, since random measurement error or environmental noise will push the length : width ratio higher than 1. In our regressions, we used the value of 1.2. With the estimated values of a, n, and b, we then estimated the frequencies of nuclei encoding for oblongness in the five parental spores using Eq. 2.