female individuals along a gradient of available soil moisture. Other data (*8, 9*) imply that the sex of individuals of some dioecious species is not irrevocably fixed genetically, but is dependent on subtle environmental variations. Our data offer no basis for determining which, if either, of these hypotheses best explains the observed tendency for the sexes to be differentially represented on sites of varying soil moisture availability.

Regardless of the causal mechanism, it seems likely that the observed environmental separation of the sexes of these wind-pollinated species increases the reproductive contribution of the average adult of either sex. Males on windy, sparsely vegetated topographic sites almost certainly contribute more genetic material to the next generation than do males on densely vegetated, depressional sites; wind is more likely to reach and effectively disperse the pollen of the former individuals. Since effective precipitation in the area of study is concentrated in the winter period (*10*) and the pollen of all species studied is dispersed within a few weeks after growth initiation, drought should not strongly depress the reproductive potential of males.

The reproductive output of females, however, is dependent on a much longer period of favorable soil moisture conditions than is that of males. Furthermore, female reproductive efforts require the largest input of resources during the driest part of the year, long after pollen production has ceased. Additionally, seed production demands the investment of many times more resources than are required for pollen production. Consequently, females on better-watered and more fertile sites can be expected to contribute more offspring to subsequent generations than females on xeric sites.

With such a differential in reproductive success of male and female individuals on xeric as opposed to more predictably mesic sites, disruptive selection (*11*) within a common gene pool seems inevitable if sex is rigidly controlled by genetics. In males, genes for maleness and tolerance of exposed, chronically droughty sites might be expected to become associated. Conversely, among females, genes conferring adaptability for better-watered sites should become associated with those for sex. Such an association of genes should make each sex competitively superior on its preferred site to the other sex and thus provide a mechanism for the observed spatial distribution of the sexes (*12*). Such differential survival of individuals would be expensive for both sexes. Control of sexual expression by environmental variation would appear to be more economical.

When the tendency for the sexes to be spatially separated arises through differential survival of seedlings, the spatial pattern can be expected to reduce intraspecific competition between reproductively active adults. The reproductive potential of dioecious species should be increased by a resource-partitioning scheme in which males occupy marginal sites and compete minimally with females on better sites (*13*). For three of the species considered here (salt grass, Mormon tea, and shadscale), male-female competition would be expected to be an important selective force, since communities dominated by these species are commonly of low diversity (indeed, salt-grass meadows approach single-species stands). Consequently, much and in some cases most of the competition these species experience is intraspecific. In at least these cases, we postulate that the adverse effects of competition between the sexes tend to complement and reinforce disruptive selection pressures arising from differential success of males and females on xeric and moist sites.

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References and Notes

1. R. Selander, *Condor* **68**, 113 (1966).
2. J. H. Brown and R. C. Lasiewski, *Ecology* **53**, 939 (1972).
3. P. D. Putwain and J. L. Harper, *J. Ecol.* **60**, 113 (1972).
4. Dioecism is a condition in which male and female sex organs are confined to separate plant individuals.
5. C. A. Black, *Soil-Plant Relationships* (Wiley, New York, ed. 2, 1968).
6. A. P. Plummer, D. R. Christensen, S. B. Monsen, *Utah Div. Fish Game Publ. No. 68-3* (1968). These authors estimate average annual precipitation for the juniper-sagebrush zone at 25 to 33 cm; for the shadscale zone, they report 23 cm/year.
7. M. V. Sivtsev and S. S. Sizov, *Biol. Nauki (Moscow)* **15** (No. 10), 78 (1972).
8. M. Arnoux and G. Mathiev, *Ann. Amelior. Plant.* **19**, 53 (1969).
9. G. G. Davidyan, *Tr. Prikl. Bot. Genet. Sel.* **42** (No. 1), 72 (1970).
10. Environmental Data Service, *Climatography of the United States No. 81-37* (Government Printing Office, Washington, D.C., 1962). Only 42 percent of the annual precipitation in north-central Utah falls during the growing season (April to September). The relative effectiveness of that moisture for plant growth is greatly reduced by rapid evaporation.
11. G. L. Stebbins, *Flowering Plants, Evolution Above the Species Level* (Harvard Univ. Press, Cambridge, Mass., 1968), p. 10. We define disruptive selection as the diversification of a homogenous gene pool into at least two distinct genotypes.
12. This type of argument also offers a possible explanation for the origin of the dioecious habit from monoecious or polygamous parental stock that is wind-pollinated. The argument need not be limited to water availability; any limiting resource that differentially affects male and female reproductive success could trigger a disruptive selection process that could eventually result in partial or complete separation of the sexes.
13. Intraspecific competition may be especially deleterious to dioecious species, since any reduction in the number or size of seeds set by the female as a consequence of competition from males will reduce not only her contribution to the next generation but that of the competing male as well (since the male's genes can be represented in the next generation only through the female).
14. We wish to acknowledge N. Negus and A. Archuleta for their assistance in gathering the data. Two anonymous reviewers of an earlier draft provided most helpful comments. Supported in part by the National Science Foundation.

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Histones and G Banding of Chromosomes

Abstract. *Polylysine, polyarginine, and histones H1, H2A, H2B, and H3 inhibit Giemsa staining and chromosome banding by binding to DNA and preventing side stacking of the positively charged thiazine dyes to the negatively charged phosphate groups on DNA. This is a nonspecific effect and does not of itself provide evidence for a role of histones in G banding. The question of whether histones are involved in chromosome banding is reviewed.*

The G banding (*1*) of chromosomes is a widely used procedure, but the mechanism is poorly understood. Since the bands correlate well with the chromomeres of pachytene chromosomes (*2*), the banding procedures are essentially enhancing a preexisting structural component of the chromosomes. The bands are produced in fixed metaphase chromosomes by various protein denaturing procedures, followed by staining with Giemsa, a mixture of thiazine dyes plus eosin. Since the treatment of chromosomes with acid to remove histones does not result in banding, but subsequent treatment with trypsin does, it has been suggested that histones are not directly involved (*3–5*). It has recently been proposed that the removal of histone fractions H1 and H2A from fixed chromosomes is involved in the induction of G bands because these fractions, when exogenously applied at low concentrations, inhibit banding (*6*). The H2B and H3 histones did not inhibit banding and were supposedly not so involved. We re-

port our examination of this phenomena.

Chinese hamster (CHO) cells were fixed in a mixture of methanol and acetic acid (3 : 1), and their chromosomes were dried in air on microscope slides. Unfractionated calf thymus histones and histone H1 were purchased from Sigma. In addition, histones H1, H2A, H2B, and H3 were prepared from whole histones extracted from frozen calf thymus nuclei by the technique of Panyim *et al.* (*7*), and fractionated on a column of Bio-Gel P-100, 100 to 300 mesh (Bio-Rad) (*8*). The fractions were pure, as assayed by Panyim-Chalkey gel electrophoresis (*9*). Histones were dissolved in 0.01*N* HCl; one volume of 0.06*M* Hepes buffer–NaOH was added, and, if necessary, further NaOH was added to *p*H 7.1. Serial dilutions were made. Histone concentrations were determined by absorbance. The histone solution (20 μl) was placed on a localized area of the microscope slide, covered with a cover slip, and incubated in a humid chamber at 25°C for 15 minutes. Four experiments plus a control (buffer with no histone) were run on each slide.

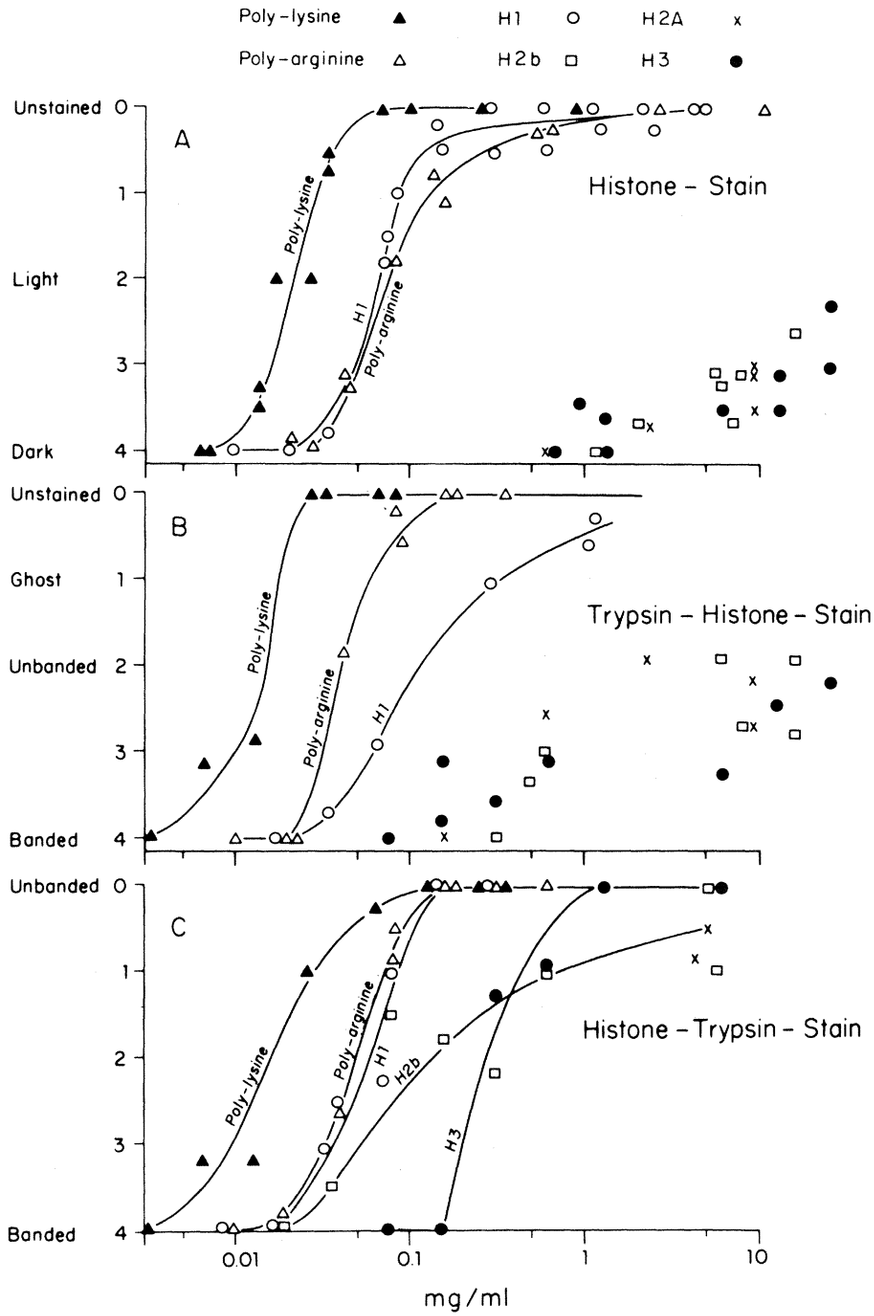

Fig. 1. (A) Microscope slides were incubated with histones at various concentrations; rinsed 4 minutes in Hepes buffer, 5 minutes in Hanks balanced salt solution, 1 minute in water at 0°C; and stained 4 minutes in 3 percent stock Giemsa, buffered to *p*H 8.5 with 0.006*M* phosphate. (B) Slides were trypsinized (0.01 percent trypsin in Hanks balanced salt solution; 2 to 3 minutes at room temperature), rinsed in 5×10^{-4}*M* phenylmethylsulfonyl fluoride (Sigma) to inactivate the trypsin, dehydrated in methanol, and dried in air before being treated as in (A). The continuum of chromosome morphology observed is shown in Fig. 2. (C) Slides were treated as described in (A), but the last 3 minutes of the washing in Hanks salt solution was in the presence of 0.01 percent trypsin.

Chromosomes were treated with various protein concentrations before they were stained. Varying the basic protein concentration produced a continuous scorable effect on the stained chromosomes. Unaffected chromosomes were arbitrarily given a score of 4, while totally affected chromosomes were given a score of 0 (Fig. 2). For each experiment, 20 individual cells were scored on coded slides. The means and standard deviations were accumulated in a calculator.

Histones and other proteins and polypeptides were tested (Fig. 1) for (i) the effect on staining of untreated chromosomes (histone treatment followed by Giemsa staining), (ii) effect on banded chromosomes (trypsin treatment, followed by histone treatment, and subsequently Giemsa staining), and (iii) effect on trypsin banding (histone treatment followed by trypsin treatment and Giemsa staining).

Polylysine, polyarginine, and all histones tested inhibit the staining of untreated chromosomes (Fig. 1). Polylysine was effective at the lowest concentrations (0.01 to 0.1 mg/ml), followed by the lysine-rich histone H1, and polyarginine. The moderately lysine-rich histones H2A and H2B and arginine-rich histone H3 required concentrations of 1 to 10 mg/ml to inhibit staining. The inhibition of G banding tends to parallel the staining inhibition curves of untreated chromosomes. This presumably indicates that histones and basic polypeptides are simply inhibiting the staining of stainable chromatin; with trypsin banded chromosomes only chromatin in the G bands is stainable. When histones are applied before trypsinization, they also inhibit banding (Fig. 1C); but here two factors are involved: inhibition of staining and interference with the action of trypsin due to the large amount of exogenously applied basic protein.

Proteins less basic than histones, such as serum albumin and cytochrome c, had no significant effect on inhibition of staining, while ribonuclease at 10 mg/ml showed a very slight effect. The fact that lysine, arginine, spermine, and spermidine showed no inhibition of staining at concentrations of up to 15 mg/ml indicates that polymerized forms of lysine and arginine are more effective inhibitors than the individual amino acids. Suppos-

edly, the individual charged groups of basic polypeptides bind cooperatively to DNA phosphates while the basic monomers do not. This would explain why thiazine dyes can more effectively compete with monomers than with polymers for DNA binding sites. When increasing amounts of NaCl were added to the H1 histone solutions, the ability of H1 histone at 0.14 mg/ml to inhibit Giemsa staining was completely abolished at concentrations higher than 0.4*M*. All these data are most simply explained by density ionic binding of the basic proteins to chromatin DNA, which is dependent on salt concentration, molecular weight, and ionic charge. This prevents access of the basic Giemsa dyes to DNA phosphate groups.

The ability of lysine-rich histone to inhibit dye binding more effectively than other histones is consistent with the observation that histone H1 is also more effective in stabilizing DNA against thermal denaturation than the arginine-rich histone (*10*). Polylysine and polyarginine, in turn, are more effective in protecting DNA from thermal denaturation than any of the histones (*11*).

The following observations suggest that histones may not be directly involved in G banding.

1) When fixed chromosomes are treated with acid for up to 4 hours to remove all histones, they still stain uniformly with Giemsa. If these dehistonized chromosomes are treated with trypsin and then stained with Giemsa, typical G bands occur (*3–5*). If chromosomes are first fixed with formalin to prevent removal of histones by a mixture of acetic acid and methanol, it is still possible to induce G banding with trypsin (*4*).

2) Inherent in proposals that histones are involved in G banding is the assumption that the histones associated with the G bands (and C bands) are different from those associated with the interbands (R bands). Several observations seem to speak against this: (i) There is a correlation between late replicating heterochromatin and G and C bands, early replicating euchromatin, and R bands (*12*). However, biochemical studies show no significant differences in the ratio of the types of histones present in heterochromatin as compared to euchromatin (*13*). (ii) The basic nu body units of chromatin appear to be composed of dimers of H2A, H2B, H3, and H4 histones (*14*), and the interaction of these histone complexes with DNA is independent of the type or source of DNA (*15*). This suggests an even distribution of histones along the chromosome. (iii) Fluorescence studies with antibodies to histones also suggest an even distribution of histones along mammalian chromatin and chromosomes (*16*).

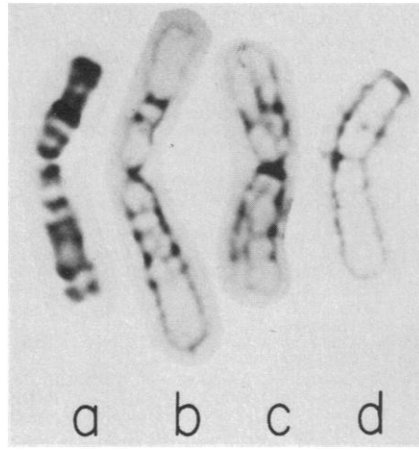

Fig. 2. Chromosomes scored in Fig. 1B. (a) Unaffected and banded, scored as 4; (b) partially banded, scored as 3; (c) slightly banded, scored as 2.5; (d) ghost cell with unstained interior, scored as 1.

3) Giemsa stains chromosomes by side stacking of the positively charged thiazine groups to the negatively charged phosphate groups (*17, 18*). At saturation, the dye-phosphate molar ratio for naked DNA is 1.0. With unfixed chromatin the ratio is 0.5 (*17, 18*), while with fixed chromatin the ratio is 0.8 (*18*), an indication that there are many free phosphate groups available for forming complexes with the dye. Adding basic protein to the fixed chromosomes binds these phosphate groups and at sufficient concentration prevents the thiazine dyes from binding. This is a nonspecific effect related to the charge density of the basic protein and does not of itself indicate that histones are involved in G banding. Equilibrium dialysis studies with methylene blue and fixed chromatin treated by conditions used in G and R banding suggest that some of the nonstaining regions are in part the result of increased binding of nonhistone proteins to DNA, thus preventing the binding of thiazine dyes (*18*).

The above points were addressed to the possibility that banding is a direct or indirect result of a different set of histones in the C and G bands compared to the R bands. A somewhat separate question is the proposal that the removal of H1 histones is necessary in order to obtain G banding (*6*). The following observations could be taken as consistent with this.

1) H1 histone was at least tenfold more effective than the other histones in inhibiting chromosome staining (Fig. 1).

2) If chromosomes were treated with trypsin (G banded, exposed to H1 or H2A, then again treated with trypsin), G banding was observed with the H1 but not with the H2A treated chromosomes (*6*).

3) H1 appears to be involved in cross-linking DNA molecules (*19*) and does not occur in the nu body (*14*). Removal of such cross-linking could be necessary before banding can be produced by subsequent trypsin or salt treatment.

4) Some reports (*4, 16, 20, 21*), but not all (*3, 22*), suggest that H1 histone is removed from the chromosomes during fixation with a mixture of methanol and acetic acid.

The suggestion that H1 must be removed before banding can be broken into two component parts: (i) H1 must be removed before the chromosomes can be stained with Giemsa, or (ii) because of its cross-linking effect H1 histones must be removed before G banding can occur.

The first part (that H1 must be removed before staining) is probably incorrect for the following reasons. (i) Hematologists routinely smear bone marrow preparations on a slide, allow them to dry, and stain with Giemsa or Wright's stain. These preparations are never exposed to conditions that would extract any histones, and yet the chromosome of mitotic cells stain intensely with these dyes. The same is true of cells in tissue culture. If they are fixed on a slide in ethanol, formalin, or glutaraldehyde, so that the histones are not removed, the metaphase chromosomes still stain intensely. Chromatin was so named because it stained intensely with basic dyes. (ii) Dye binding studies show that whole chromatin, with its full complement of histones, binds 0.5 mole of thiazine dye per mole of DNA phosphate, and this is more than adequate to stain the chromatin (*17, 18*).

The second part, that the cross-linking effect of H1 histones must be removed to obtain banding, is not readily ruled out. Consistent with this is the observation that whole mount electron microscopy of chromosomes fixed in methanol and acetic acid shows no banding while electron microscopy of trypsin-treated, methanol-acetic acid–fixed chromosomes shows banding in the absence of any Giemsa staining (*23*). Since the electron beam responds only to density, this is strong evidence that some rearrangement of the chromatin has occurred to reveal the underlying chromomere pattern (*2*) of the chromosomes. There are two observations, however, that suggest that H1 cross-linking is not involved in this process. (i) If all the histones are extracted with acid, the chromosomes still stain

uniformly. If H1 cross-linking played a significant role one would anticipate that some rearrangement of chromatin and banding would occur when this histone was removed. If the chromosomes are then treated with trypsin, banding is produced (*3–5*). (ii) G banding can be produced in chromsomes where all the histones have been retained by prior fixation in formalin (*4*).

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References and Notes

1. Paris Conference (1971), *Birth Defects, Original Article Series* (The National Foundation) **8**, 7 (1972).
2. T. A. Okada and D. E. Comings, *Chromosoma* **48**, 65 (1974); M. A. Ferguson-Smith and B. M. Page, *J. Med. Genet.* **10**, 282 (1973); D. E. Comings and T. A. Okada, *Exp. Cell Res.* **93**, 267 (1975).
3. D. E. Comings and E. Avelino, *Exp. Cell Res.* **86**, 202 (1974); D. E. Comings, *ibid.* **67**, 441 (1971).
4. A. Sivak and S. R. Wolman, *Histochemistry* **42**, 345 (1974).
5. S. Matsukuma and T. Utakoji, *Exp. Cell Res.* **97**, 297 (1976).
6. R. L. Brown, S. Pathak, T. C. Hsu, *Science* **189**, 1090 (1975).
7. S. Panyim, D. Bilek, R. Chalkley, *J. Biol. Chem.* **245**, 4206 (1971).
8. K. R. Sommer and R. Chalkley, *Biochemistry* **13**, 1022 (1974).
9. S. Panyim and R. Chalkley, *Arch. Biochem. Biophys.* **130**, 337 (1969).
10. R. C. Huang, J. Bonner, K. Murray, *J. Biol. Chem.* **8**, 54 (1964); L. S. Hnilica and D. Billen, *Biochim. Biophys. Acta* **91**, 271 (1964).
11. T. Y. Shih and J. Bonner, *J. Mol. Biol.* **48**, 469 (1970).
12. D. E. Comings, *Adv. Hum. Genet.* **3**, 237 (1972); *Birth Defects, Proceedings of the 4th International Conference* (Excerpta Medica, Amsterdam, 1974), p. 44.
13. ———, *J. Cell Biol.* **35**, 699 (19 C. Johmann, R. A. Eckhardt, M. A. Gorovsky, *ibid.* **58**, 119 (1973).
14. J. A. D'Anna and I. Isenberg, *Biochemistry* **13**, 2098 (1974); R. D. Kornberg and J. O. Thomas, *Science* **184**, 865 (1974); R. C. Hardison, M. E. Eichner, R. Chalkley, *Nucleic Acid Res.* **2**, 1751 (1975); S. C. Elgin and H. Weintraub, *Annu. Rev. Biochem.* **44**, 725 (1975).
15. R. Axel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4101 (1974).
16. M. Bustin, H. Yamasaki, B. Goldblatt, M. Shani, E. Huberman, L. Sachs, *Exp. Cell Res.* **97**, 440 (1976); M. Bustin, D. Goldblatt, R. Sperling, *Cell* **7**, 297 (1976).
17. R. F. Itzhaki, *Biochem. J.* **122**, 583 (1971); R. J. Clark and G. Felsenfeld, *Nature (London) New Biol.* **229**, 101 (1971); D. E. Comings, *Chromosoma* **50**, 89 (1975).
18. D. E. Comings and E. Avelino, *Chromosoma* **51**, 365 (1975).
19. L. S. Hnilica, *The Structure and Biological Function of Histones* (CRC Press, Cleveland, Ohio, 1972).
20. T. Brody, *Exp. Cell Res.* **85**, 255 (1974); A. T. Sumner, H. J. Evans, R. A. Buckland, *ibid.* **81**, 214 (1973).
21. L. Pothier, J. F. Gallagher, C. E. Wright, P. R. Libby, *Nature (London)* **255**, 351 (1975).
22. C. Dick and E. W. Johns, *Exp. Cell Res.* **51**, 626 (1968).
23. G. D. Burkholder, *ibid.* **90**, 269 (1975).
24. We thank Dr. B. J. Conner for isolating the histones. Supported by NIH grant GM 15886 and NIH fellowship GM 05165. Request reprints from D.E.C.
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On *Palaeosclerotium* as a Link Between Ascomycetes and Basidiomycetes

Dennis (*1*) suggests that a fossil *Palaeosclerotium* is a link between the ascomycetes (Ascomycota) and basidiomycetes (Basidiomycota). He does not provide a convincing case that this organism is intermediate between these two fungal groups. Septal pore swellings are illustrated and interpreted as dolipores characteristic of septa of dikaryotic hyphae of some basidiomycetes. However, septal pore swellings are also found in some zygomycetes, ascomycetes, imperfect fungi, and monokaryotic hyphae of many basidiomycetes (*2*), which makes it hazardous to interpret these structures as characteristic of basidiomycetes. What is unique to many members of the latter group is the septal pore cap, a modified form of endoplasmic reticulum which surrounds the septal pores and is unlikely to be preserved in the fossil record. Although the nonascogenous hyphae in the ascocarp may lack septal pore swellings (*3*), their presence has been demonstrated in vegetative hyphae of an imperfect fungus with ascomycetous affinities (*2*). The septal pore swellings of basidiomycetes are probably not a part of the cross wall but resemble vacuoles, and they are absent in dead cells (*4*). Thus they may be absent from fossilized basidiomycete septa.

The author points out that species of the ascomycete *Tuber* are reported to bear clamp connections. However, neither the presence of clamp connections in *Tuber* nor the analogy between crosiers of the ascomycetes and clamp connections of basidiomycetes has been fully accepted (*5*). The presence of a free-living dikaryophase in a complex organism like *Palaeosclerotium* would be most unusual, as it is absent from all but a few simple ascomycetes, but is typical of basidiomycetes.

Palaeosclerotium could represent more than one organism, as noted by Dennis. He interprets figure 1C (*1*) as demonstrating an organic connection of clamp-bearing hyphae to the fruiting body, which is crucial to his argument, but this is not clear in the micrograph. Interpretation of this fossil as possessing features of both these fungal groups must be viewed with caution.

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References

1. R. L. Dennis, *Science* **192**, 66 (1976).
2. A. Beckett, I. B. Heath, D. J. McLaughlin, *An Atlas of Fungal Ultrastructure* (Longmans, London, 1974); F. C. Terracina, *Can. J. Bot.* **52**, 2587 (1974).
3. J. S. Furtado, *Mycologia* **63**, 104 (1971).
4. E. C. Setliff, W. S. MacDonald, R. F. Patton, *Can. J. Bot.* **50**, 2559 (1972); C. Thielke, *Arch. Mikrobiol.* **82**, 31 (1972); C. E. Bracker and E. E. Butler, *Mycologia* **55**, 35 (1963); K. Wells, *ibid.* **56**, 327 (1964); Y. Nakai and R. Ushiyama, *Rep. Tottori Mycol. Inst.* **11**, 7 (1974).
5. D. B. O. Savile, in *The Fungi*, G. C. Ainsworth and A. S. Sussman, Eds. (Academic Press, New York, 1968), vol. 3, pp. 649–675; R. L. Shaffer, *Mycologia* **67**, 1 (1975).

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