Reassignment of Gymnomycota

Whittaker's five-kingdom classification (*1*) of living organisms is possibly the most satisfactory yet proposed. It should provide not only a more sensible system of classification but also a more logical means of presenting this subject to students.

With the object of strengthening rather than criticizing the proposal, I recommend an alteration in treatment of a group with which I am familiar. The change would be in line with Whittaker's emphasis on mode of nutrition. It is unlikely that any members of the subkingdom Gymnomycota, which (excepting labyrinthulids) ingest their food, are related to what are commonly considered the true fungi, which have absorptive nutrition. Therefore, I suggest that this group be transferred to the kingdom Protista, possibly as a subkingdom, with the bulk of the protists in a separate subkingdom. It might then be expedient, at least tentatively, to include the plasmodiophorids and protomyxids (plasmodial forms living in the cells of other organisms) in the Gymnomycota. The latter are known to ingest, and there is evidence that the former may ingest as well as absorb their nutrients.

Whittaker's statement that his three phyla of Gymnomycota have probably had three separate phylogenetic origins is not supported by our recent findings (*2*) on a still simpler and more primitive group, the protostelids, which are clearly allied with the Gymnomycota. Evidence has been presented in support of the origin of a major portion of the cellular slime molds from the protostelids, and there is also a fair possibility that plasmodial slime molds may have had their origin in this group. In addition, there are reasons for believing that the cellular slime molds are not a monophyletic group (*3*).

Finally, there are probably more forceful arguments for allying the hyphochytrids, which have absorptive nutrition, with the aquatic fungi rather than the protists. Though their origin may have been independent of other groups of fungi, a good case can be made for their derivation from the laterally biflagellate fungi (Oomycota) through loss of the posterior flagellum.

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I consider Dr. Olive's suggested treatment of the Gymnomycota as a subkingdom of the Protista an acceptable alternative to my own arrangement. The mittenlike form of the kingdom Fungi in one of my figures is intentional: in my classification and some others the slime molds stick out like a sore thumb. There are, necessarily, other questions on my classification of the fungi; among these questions are the separation of the hyphochytrids from other fungi mentioned by Dr. Olive and the degree of evolutionary separation implied for the subkingdoms Dimastigomycota and Eumycota. I feel my treatment of the fungi is reasonable but by no means definitive, and I shall watch with interest for the conclusions the mycologists themselves reach from considering classification of fungi in contexts different from the traditional plant kingdom.

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Visual Form Discrimination on the Basis of Relative Distribution of Light

Winans (*1*) has reported that cats with lesions of the visual cortex can discriminate inverted from upright triangles but has cautioned that her subjects may have responded to the spatial distribution of light rather than to the form of the stimuli per se. Dodwell and Freedman (*2*) have reiterated this possible interpretation but offer no relevant data. Nor are there pertinent observations in the classical literature (*3*). In the course of a study unrelated to this question I have recorded some data with normal rats which suggest that a triangle discrimination based on spatial distribution of luminous flux is quite possible.

Eight rats which had previously learned to avoid shock by choosing either an inverted or an upright white triangle on a black background were given a free choice on ten consecutive trials between the same forms but with black and white areas interchanged; that is, they chose between an inverted and an upright black triangle on a white background. The triangle forms were shifted from one side of the choice point to the other according to a modified Gellerman (*4*) series. This was accomplished by rotating the stimulus cards 180° so that choice on the basis of olfactory cues from earlier choices is unlikely.

Note that the same forms (for example, upright white and upright black triangles) have opposite flux gradients from top to bottom, while opposite forms (for example, upright white and inverted black triangles) are similar in this respect. If the original discrimination were mainly on the basis of form, one would expect the animal to choose the new stimulus having within it the precise contours of the previously positive stimulus, oriented the same way (for example, going from an upright white triangle to an upright black triangle). If the original discrimination were mainly on the basis of relative distribution of light, one would expect the animal to disregard the contour and orientation cues and choose the stimulus with similar black-white gradients (for example, going from an upright white triangle to an inverted black triangle).

The data support the latter hypothesis. Seventy-three of the 80 possible choices were to the opposite form. This preference is statistically different from random choice ($\chi^2 = 29$, d.f. $= 1$; $P < .001$). All animals chose the opposite form on at least seven of the ten trials and half the subjects chose it every time. Figure 1 shows the percent of choices to each transfer stimulus from the original training stimuli.

Although one cannot conclude from these results with rats that Winans' cats were discriminating on the basis of spatial distribution of light, the pos-

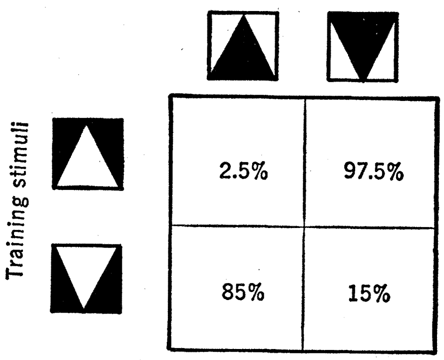

Fig. 1. Percent of choices to each transfer stimulus from the original training stimuli.

sibility has some empirical support. As Winans pointed out, judgments on the basis of luminous flux can be made by animals with visual-cortex damage (*5*). Reducing the size of the figure as in the Winans study is not an unequivocal test, since the subjects may have learned to attend selectively to smaller black-white gradients. A simple control test such as the one described here gives an indication of whether the discrimination is based on form or on relative distribution of light within the figure.

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Buchtel's study with normal rats does not bear directly upon the major conclusion from my report—that, contrary to indications from previous studies, striate decorticate cats are capable of discriminating stimuli equated for total luminous flux (*1*). However, Buchtel's data are particularly relevant to a second consideration which I raised in that report concerning the possible cues for discrimination of the stimuli. The erect and inverted triangles which have been used in classical and recent studies of visual form discrimination (*2*) display localized flux cues on corresponding restricted regions of the stimuli, as well as flux gradients, either of which could facilitate the discrimination of these stimuli by animals capable of discriminating regional flux differences within areas equated for the total flux.

As I indicated in my reply to Dodwell and Freedman (*3*), after the striate decorticate cats had mastered the series of triangle discriminations, the cats were presented with a number of transfer tests. Among those tests was a black-white figure-ground reversal similar to that described by Buchtel. When three of the striate decorticate cats were presented with black triangles (7.62 cm high and 4 cm at the base) centered on lighted panels (21.6 cm by 11.4 cm) for 40 differentially reinforced trials (erect triangle rewarded, as in discrimination training), the correct responses averaged 53.3 percent. Similarly, unoperated cats which had mastered the same series of triangle discriminations responded to this test with an average of 50.6 percent correct responses over the 40 test trials. Unlike Buchtel's rats, the cats did not prefer the inverted triangle. However, in Buchtel's experiment, the rats were rewarded regardless of whether they chose the erect or inverted triangle, whereas in my study, the cats were rewarded only for selecting the erect triangle. This differential reinforcement may have counteracted a preference for the inverted triangle and resulted in the apparent absence of differential responses.

The results of this black-white figure-ground reversal test with the cats support the conclusion that these animals were dependent upon flux gradients or localized flux cues for discrimination of the triangles. But, in other transfer tests (in preparation for publication) these same lesioned and unoperated cats chose the erect triangle on critical trials (food behind both doors) when the triangles were presented in outline form and in outlines with the bases deleted. In these figures the flux gradients were greatly reduced and the localized flux cues were altered. Thus, if the striate decorticate cats relied on flux gradients or localized flux cues to discriminate the triangles, they were capable of utilizing surprisingly subtle cues of these types to guide their discrimination behavior.

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Are Honeybees Deficient in Phosphomannose Isomerase?

Sols, Cadenas, and Alvarado (*1*) have interpreted mannose toxicity in honeybees as a metabolic disease characterized by a deficiency in phosphomannose isomerase, presumably caused by a mutational loss of the bee's ability to synthesize this enzyme. Their conclusions were based on experiments with homogenates of honeybees prepared in 5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.0. Since phosphomannose isomerase, isolated from brewers' yeast (*2*), is a zinc-metalloenzyme (*3*), the question arose of whether this apparent lack of phosphomannose isomerase (*1*) is in fact a deficiency, or whether it was perhaps caused by the chelating agent in the extraction medium. We have, therefore, made measurements of phosphomannose isomerase activities in honeybee extracts prepared and stored under a variety of conditions in the absence or presence of chelating agent.

Conditions for extraction of the enzyme were chosen to resemble as closely as possible those described (*1*). Normal worker honeybees (*4*) were fasted for 1 hour and killed by freezing at -18°C; they were stored frozen until use. Portions (5 to 10 g) of the frozen bees were ground for 5 minutes, with twice the amount of buffer, in a chilled mortar; the suspension was centrifuged at 1°C for 20 minutes at 29,000g. The supernatant was poured through glass wool to remove the pollen and was assayed immediately to obtain the initial phosphomannose isomerase (PMI) activity. The extracts were then incubated at 30°C or kept frozen at -15°C. Extractions were made either in 0.1M piperazine bis-ethanesulfonate (Na^+) buffer, pH 7.15, selected for its nonchelating property (*5*), or in 5 mM EDTA, pH 7.0, as used by Sols *et al.* (*1*). Phosphomannose isomerase was assayed at 30°C through coupling to phosphoglucose isomerase and glucose-6-phosphate dehydrogenase, by a continuous spectrophotometric method (*2*). Phosphoglucose isomerase and hexokinase (with glucose as substrate) were also measured by coupling to the glucose-6-phosphate dehydrogenase system. For all enzyme measurements, emphasis was placed on determining true initial velocities.

When the phosphomannose isomerase activities of various bee preparations were compared, extraction with the piperazine-bis-ethanesulfonate buffer always yielded higher PMI activities (5.9 ± 0.6 μmole of substrate converted per 15 minutes per gram of bee; seven preparations) than extraction with EDTA (4.1 ± 0.7 μmole units, as above; three preparations). The values for the piperazine-bis-ethanesulfonate extracts are about six times higher than the average PMI ac-