Pavlova and the taxonomy of flagellates, especially the Chrysomonads

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SUMMARY

This thesis is concerned with the cell architecture of the flagellate genus *Pavlova*. This genus of small unicellular organisms plays a role in a controversy concerning the taxonomy of the yellow, gold-coloured, and brown flagellates that have long been classified together under the name of Chrysomonads. Within this group two sub-groups can be distinguished, which are considered by a number of authors to be separate classes, the Chrysophyceae and the Haptophyceae. An argument against making this subdivision of the Chrysomonads is that the genus *Pavlova* appears to show characteristics of both sub-groups.

Electron-microscopic research was undertaken on four species isolated for the purpose in order to make it possible to provide a definitive statement in the controversy mentioned. At the beginning of this investigation only two species were known and no data were available on their internal structure.

The structures from which it can be seen to which of the two Chrysomonad classes an organism belongs are described and illustrated in the first chapter. These structures are:

1. The flagellar hairs, that in Chrysophyceae consist of three parts.
2. The haptonema, a special type of protoplasmic extension, that occurs in Haptophyceae and was previously regarded as a flagellum.
3. Scales that cover the cell body and that consist of silica in the Chrysophyceae and of cellulose and pectin-like material, and sometimes also of calcium carbonate in the Haptophyceae.
4. The pattern of the photosynthetic lamellae in the plastids, each consisting of three parallel thylakoids. One lamella curves around the other lamellae in the Chrysophyceae, but not in the Haptophyceae.

The mutual similarity in structure of the Haptophycean scales is not immediately clear. In this thesis a number of representative types are therefore compared. The main structural similarity is found in the pattern of the cellulose fibrils of the skeleton of the scales. In one layer which can usually be observed, the fibrils run approximately radially, in such a way that four quadrants become visible in the scale.

In the second chapter, a discussion is presented on how much was known in the first half of this century about the structures described in the first chapter, and to what extent this knowledge was useful for the taxonomy of the Chrysomonads. Apart from scales of silica and calcium carbonate these structures could not be observed to a sufficiently detailed extent to characterize the classes. Flagellates covered with scales of calcium carbonate were indeed excluded from the Chrysophyceae by Pascher in 1914 (Über Flagellaten und Algen. Ber. Dtsch. Bot. Ges. 32: 136-160). Silica scales occur only in some Chrysophyceae. The argument for the mutual affinity of all Chrysophyceae was found in the urn-shaped cysts with their intracellularly formed silica walls.

Subsequently in the same chapter current ideas on the taxonomy of the Chrysomonads are discussed against the background of the introduction of electron-microscopic techniques. The features discovered with the aid of the electron microscope are controllable in almost all cases, in contrast to the cysts, the formation of which can hardly be induced if at all. The electron-microscopic features have therefore supplanted the cysts in the taxonomic discussions.

Four chapters report on the research on the cell architecture of the four *Pavlova* species. These species were isolated by repeated selective pipetting from crude cultures obtained by inoculating seawater media with field material. Shadow preparations, sections, freeze-etch preparations and negatively stained preparations were investigated using the electron microscope. This was supplemented by the observation of living cells using the light microscope. Reagents such as iodine-potassium iodide, zinc chloride iodine-potassium iodide, Congo red, brilliant cresyl blue, toluidin blue, ruthenium red and Janus green were used to demonstrate in situ cell components such as reserve products, cell-wall material and mitochondria.

For each species an argument is presented for its inclusion in the *Pavlova* genus, and it is indicated how the study of the species concerned has led to the closer precision and modification of our conception of the genus. The *Pavlova* genus does not constitute a link between the two classes of Chrysomonads. It belongs to the class of Haptophyceae, although it occupies a special position in this class owing to:

1. The asymmetrical flagellar apparatus.
2. Paramylon as a reserve polysaccharide.
3. Small particles of acid polysaccharide, that usually cover one of the flagella and often the cell body too. Typical Haptophycean scales are lacking.

The extracellular particles of acid polysaccharide are formed in the Golgi apparatus and are released from the cell via a deep invagination in the cell. The system of flagellar roots contains microtubuli and microfilaments, but no large cross-striated root. Under the cell surface there is a cisterna of endoplasmic reticulum in which a thin layer of cellulose is present. That part of the cell situated outside this cisterna can be cast off, as a result of which the membrane separating the cisterna from the rest of the cell becomes the new plasmalemma. During cell division cleavage does not commence from the cell surface, but from the above-mentioned cisterna. In most species the cells can surround themselves with a capsule of slime, that after various cell divisions forms the matrix of colonies, and in which remains of the cast-off outermost layer of the cells are present.

In the penultimate chapter the mutual relationships are determined between *Pavlova*, the symmetrical Haptophyceae, the Chrysophyceae and a number of other important groups of flagellates, by means of Jaccard's similarity coefficients. Here the differences in complexity of the characteristics are taken into consideration. In the first instance characteristic species of each of the taxonomic groups selected are compared. Subsequently traits that do not occur in all representatives of the taxonomic

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group are omitted, and in this way a clearer picture of the relationships is gained. The Haptophyceae, at least the representatives of this class without scales or a haptonema, evidently occupy an intermediate position between the Chrysophyceae and the Prasinophyceae, a class of green algae. This numerical approach also shows that the *Pavlova* genus is clearly related to the symmetrical Haptophyceae, but less clearly to the Chrysophyceae. It therefore forms no link between the Chrysophyceae and the symmetrical Haptophyceae. The relationships established are compared with recent taxonomic systems.

In the last chapter the concept of 'species' is discussed. The way in which the description of the species has arisen determines the meaning of the word 'species'. It is not possible to observe all characteristic features of individual cells either with the light microscope or the electron microscope. This is the case with living cells because the orientations required for the observation of the various features are not the same. Most staining techniques and all usual electron-microscopic techniques destroy part of the information in order to make another part observable. The description of a species is a synthesis of information on details of different individuals, about each of which very little is known. The concept of 'species' with respect to *Pavlova* and other recently described flagellates corresponds to the reconstructions of fossil plants.