eny of the genus. The possibility that such groupings also may be indicative of systematic relationships among *Pichias* is suggested by the fact that homothallic species of *Pichia* occur within each of the three sorts of groups whereas the known, purely heterothallic species exhibit only large PR sectors.

2. The non-photoreactivating, primitive species of *Hansenula* ferment few sugars and the majority of such species grow vegetatively only in haplophase; the strongly photoreactivating, advanced species are more versatile fermentatively and, typically, grow vegetatively either as haplophase-diplophase mixtures or in diplophase exclusively[2,5]. Analogous correlations are noted with the *Pichias*. Though species of *Pichia*, collectively, have a much more restricted range of fermentative capabilities than the *Hansenulas*, KREGER-VAN RIJ has compared all species of *Pichia* for abilities to ferment four common sugars[7]. The Table indicates that the nonphotoreactivating species of *Pichia* ferment an average of 1.4 of these sugars each, whereas species having large PR sectors ferment an average of 2.0 sugars each; furthermore, 64% of the non-photoreactivating species but only 30% of the species having large PR sectors grow vegetatively in haplophase, exclusively.

3. Proton magnetic resonance spectra of the cell wall mannans of the *Hansenulas* have established that the weakly photoreactivating species (PR sector, 0.1) belonging to line 5 of WICKERHAM's phylogeny and the non-photoreactivating progenitor of that line, *H. wickerhamii*, possess a unique mannan structure which distinguishes them from other members of the genus[3,4]. This same distinctive structure has been identified in the cell wall mannans of the following species of *Pichia*[3]: *P. angophorae, P. fermentans, P. fluxuum, P. kluyveri, P. membranaefaciens, P. pinus, P. terricola, P. trehalophila.* The Table reveals that these particular species of *Pichia*, like their *Hansenula* counterparts, either do not photoreactivate or have PR sectors of 0.1.

These observations suggest that PR sectors constitute an additional parameter of affinity between the genera *Hansenula* and *Pichia* and may serve as useful data in the eventual resolution of phylogenetic relationships among species of *Pichia*.

Zusammenfassung. Es wird der Beweis erbracht, dass photoreaktivierungsfähige Sektoren von Zellen einen zusätzlichen Affinitätsparameter zwischen Hefen der Gattungen *Hansenula* und *Pichia* bilden und vielleicht wertvolle Daten für die endgültige Erschliessung der intragenerischen Phylogenie der *Pichia* liefern.

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COGITATIONES

Flow in the Phloem and the Immobility of Calcium and Boron: A New Hypothesis in Support of an Old One

The sieve tubes of the phloem of plants are the conduits through which photosynthate and additional, quantitatively minor solutes are distributed from the leaves and other 'sources' to 'sinks' – regions where these nutrients are utilized, including the roots. The most widely held view of the mechanism of movement in the sieve tubes is the pressure flow hypothesis of MÜNCH; see CRAFTS and CRISP[1]. According to this view, a mass flow of water and dissolved solutes moves through the lumen of the sieve elements, and from one such element to the next through the connecting pores which are clustered in sieve areas of the cell walls between contiguous sieve elements abutting end to end.

For such a flow to occur the interior of the sieve elements and the pores connecting them must be relatively open; the numerous membrane-bound organelles present in other types of cells would tend to plug up the conduit. The vacuole, in particular, would represent an obstacle to any free flow. In mature cells not specialized for conduction it occupies the bulk of the volume of the cell, restricting the cytoplasm to a thin layer between the plasmalemma, the outer membrane which lies appressed against the cell wall, and the tonoplast – the membrane between the cytoplasm and the central vacuole.

In accord with expectations based on the pressure flow hypothesis, the interior of the mature sieve element is indeed devoid of much of the cytoplasmic apparatus which would impede a longitudinal flow of a solution. The tonoplast (and hence a true vacuole) is absent, and so is the nucleus. The relatively few membranous stuctures remaining (mitochondria, plastids, endoplasmic reticulum) usually lie near the plasmalemma, along the wall, leaving a relatively structureless lumen within, which appears to offer minimal resistance to the longitudinal flow envisioned by the MÜNCH hypothesis.

Through the sieve tubes move not only sugars but also inorganic nutrients including potassium, magnesium, phosphate, and others[1,2]. But curiosly, concentrations of calcium and boron in the sieve tubes are very low, and so, as a result, are the quantities of these nutrients moved in the phloem[1,2].

The relative immobility of calcium and boron in the phloem is disadvantageous or non-adaptive in that it may cause local deficiences resulting from their failure to be redistributed, via the phloem, from regions where their concentrations are high, as in older leaves, to young, actively growing regions[2]. I offer the following hypothesis to explain the paradox of an adaptation which is seemingly disadvantageous.

Calcium. This nutrient is essential for the functional and structural integrity of plant cell membranes[2,3]. In calcium deficient cells there is 'a general disintegration of the cell contents'[4], 'structureless areas' appear and extend progressively, and there ensues a 'disintegration of the various membranous structures'[5]. These observations on calcium deficient cells are quite parallel to those made by students of phloem structure concerning the contents of sieve tubes – cells known to contain very little calcium. I

[1] A. S. CRAFTS and C. E. CRISP, *Phloem Transport in Plants* (W. H. Freeman, San Fransisco 1971).
[2] E. EPSTEIN, *Mineral Nutrition of Plants: Principles and Perspectives* (John Wiley and Sons, New York 1972).
[3] R. G. W. JONES and O. R. LUNT, Bot. Rev. *33*, 407 (1967).
[4] H. SOROKIN and A. L. SOMMER, Am. J. Bot. *27*, 308 (1940).
[5] N. G. MARINOS, Am. J. Bot. *49*, 834 (1962).

therefore propose that exclusion of calcium from sieve tubes is part of the developmental process whereby they attain the relatively structureless condition which presumably fits them for the function of longitudinal conduction of a flowing solution. The characteristic immobility of calcium in the phloem, with the attendant inefficient distribution of this nutrient in the plant, is on this view the evolutionary price paid for the adaptation of sieve tubes as relatively open, unstructured conduits through which a solution of sugars and other nutrients is free to flow. The one membrane which is fully functional – the plasmalemma – abuts on the cell wall and is therefore exposed to calcium present in the extracellular space or 'apoplast'.

Boron. Like calcium, boron is an essential nutrient; a deficiency of it has prompt and often disastrous effects on growing points and hence, on the entire plant. Its failure to be redistributed via the phloem is apparently disadvantageous. However, I propose that a deficiency of it in sieve tubes, like calcium deficiency, is connected with the role of sieve tubes as conducting elements. The pores connecting sieve tube protoplasts through the intervening walls are often lined with a sleeve of callose which tends to narrow these openings. Recent evidence[1] is to the effect that unless sieve tubes are injured, the sleeves of callose are not sufficiently thick to cause a critical narrowing of the pores. However, various injurious agents including borate[6] cause callose to be deposited more copiously than is otherwise the case, narrowing the pores and in extreme cases, blocking them completely. Like exclusion of calcium, therefore, exclusion of boron from sieve tubes may be an adaptation favoring their maintenance as open conduits.

Part of the above hypothesis (that concerning calcium) has been briefly referred to before[2].

Résumé. Contrairement à la plupart des éléments nutritifs, le calcium et le bore sont en grande partie exclus des tubes criblés et par conséquent ne sont pas efficacement distribués dans la plante, ce qui est apparemment désavantageux. On suppose que l'exclusion de ces deux éléments des tubes criblés favorise leur maintien comme conduits ouverts.

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[6] W. ESCHRICH, H. B. CURRIER, S. YAMAGUCHI, R. B. MCNAIRN, Planta *65*, 49 (1965).

[7] I thank ALDEN S. CRAFTS and H. B. CURRIER for reviewing the manuscript.

PRO EXPERIMENTIS

A Method for Freezing Living Ovaries of *Drosophila melanogaster* Larvae and Its Application to the Storage of Mutant Stocks

The isolation of *Drosophila* mutants has been hindered by the fact that no satisfactory method is available for preserving living flies, so that the stocks have to be maintained constantly.

A method for freezing imaginal disc cultures of *Drosophila* in liquid nitrogen has been reported[1], but it gave variable results when applied to discs taken directly from the larva. Therefore, a new technique for freezing imaginal discs was developed which gives complete survival of the tissue[2]. Encouraged by these results we adapted this method to the freezing of larval ovaries. Transplanted ovaries can establish a connection with the host's oviduct and give rise to offspring[3]. In this preliminary note we report on a method for freezing larval ovaries which can be used for preserving mutant stocks.

The ovaries were dissected from wildtype donor larvae of the late third instar (110 h after egg deposition, at 25 °C) in a drop of balanced saline solution[4], and the adhering fat body was carefully removed by tungsten needles. A dozen ovaries was collected and transferred into a 2 ml ampoule containing 0.5 ml of freezing medium I, which consists of 5% glycerin, 80% Schneider's *Drosophila* medium[5] and 15% bovine serum album in fraction V (2 mg/ml). It proved to be most convenient to transfer the ovaries in a small depression slide[6] (2 mm in diameter, 0.2 mm deep, gold (95%) – nickel (5%) which was dropped directly into the ampoule. This prevents dilution of the medium and loss to the tissue. The ovaries are incubated in freezing medium I for 2 h at room temperature. Afterwards, 0.5 ml of freezing medium II (15% glycerin, 70% Schneider's *Drosophila* medium, 15% bovine serum albumin fraction V [2 mg/ml]) are added, the media are mixed gently, and the ampoule is sealed.

Survival and fertility of frozen ovaries

Experiment	1	2	3	4	5
Number of ovaries frozen	12	12	12	12	12
Number of ovaries re-implanted	11	10	8	11	10
Number of ovaries developing normally	6	6	7	8	3
Number of ovaries reduzed in size	1	2	—	2	4
Number of ovaries with progeny	5	4	4	6	5
Total number of progeny from donor ovaries	27	32	12	31	27
Total number of progeny from host ovaries	110	130	116	161	159

[1] E. GATEFF and H. SCHNEIDERMAN, A. Rep. Lab. Schneiderman, Case Western Reserve University (1968), p. 74.

[2] W. BRÜSCHWEILER, in preparation.

[3] B. EPHRUSSI and G. BEADLE, Bull. biol. Fr. Belg. *69*, 492 (1935).

[4] L. CHAN and W. GEHRING, Proc. Nat. Acad. Sci. USA *68*, 2217 (1971).

[5] Schneider's Drosophila Medium (revised), Grand Island Biological Comp.

[6] Depression slide used for 'freeze-etching', Balzers AG, 9496 Balzers Principality of Liechtenstein.