ELECTROPHORETICALLY DETECTABLE VARIATION OF PROTEIN FRACTIONS IN THE DIATOM *Asterionella formosa* HASSAL

By

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ABSTRACT

The present study consisted of an examination of the population structure and a survey of interpopulation variation in the common freshwater planktonic diatom, Asterionella formosa Hassal, by means of disc electrophoresis.

One hundred and ten clones from 25 North American and seven European populations (bodies of water) were isolated or obtained from other investigators. Twenty populations were represented by two or more isolates obtained either simultaneously or at different times of the year.

Soluble proteins, present in crude extracts of homogenized cells from batch cultures of each isolate, were separated in polyacrylamide gels and stained either with a non-specific protein stain or for enzyme activity. Six enzymes (SAP, AlP, GDH, MDH, AcP, and EST) were examined but only the latter three, together with general protein, showed variation in electrophoretic banding patterns.

There was no detectable variation (with one possible exception) among multiple isolates from the same population, suggesting that the populations were clonal or at least highly homogeneous genetically. This was true both for contemporaneous isolates and for isolates obtained at different times of the year and in different years.

On the other hand, isolates from different populations differed by three to 28 bands out of 41. The frequency distribution of a measure of dissimilarity between pairs of population-specific electrophoretic patterns is unimodal, indicating that the observed variation is essentially continuous and that the collection of isolates is not comprised of a small number of discrete races. However, identification of groups of populations by means

of hierarchical cluster analysis in terms of their geographical origin and of the ecological characteristics of their habitat was possible only to a limited extent.

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Finally, I am grateful to my wife, Helen, without whose assistance and encouragement this study would never have been completed.

LIST OF ABBREVIATIONS

AcP = acid phosphatase

AlP = alkaline phosphatase

EST = esterase

GDH = glutamate dehydrogenase

LAP = leucine aminopeptidase

MDH = malate dehydrogenase

Bis = N,N'- Methylenebisacrylamide

EDTA = (Ethylenedinitrilo) tetraacetic acid

NAD = nicotinamide - adenine dinucleotide

PMS = phenazine methyl sulfate

TEMED = N,N,N',N' - Tetramethylethylenediamine

TRIS = tris (hydroxymethyl) aminomethane

K_s = Michaelis-Menten half-saturation constant

R_f = electrophoretic mobility of a protein fraction with respect to mobility of marker dye expressed as a percentage

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INTRODUCTION

Diatom taxonomy is based on the morphological species concept, individual species being defined by the structural features of their silica frustules. This approach, common to both extant and fossil species, is largely unavoidable because only a small percentage of the more than 10,000 living species has ever been cultured (Eppley, 1977).

It is not known to what extent morphologically defined diatom species correspond to biological species that are delimited through reproductive isolation. Likewise, it is not known whether the biological species concept applies to all diatoms, since sexuality in several of the better-known diatoms is yet to be demonstrated (Drebes, 1977). Conspecificity of different isolates may be difficult to prove even in cases when sexuality can be induced in the laboratory, because many diatoms are homothallic (Drebes, 1977). In such cases more complicated methods, such as induction of mutants followed by complementation tests, may have to be used (Beam and Himes, 1977).

Our understanding of the basis of variation in morphological characters; i. e. whether environmental, size-dependent, or genetic; is often limited. There are many examples of clonal diatom cultures producing valve structures characteristic of more than one species or genus (see Guillard and P. Kilham, 1977), thus revealing the arbitrary nature of the present taxonomic system.

Morphological characteristics may by themselves be insufficient to distinguish between pairs of sibling species, geographical races, or ecotypes because not all genetic differentiation results in morphological differentiation. This is true particularly of small and structurally simple organisms such as the ciliates (Borden et al, 1977).

The advent of protein electrophoresis some 15 years ago has placed a powerful new tool in the hands of systematists, enabling them to bypass little-understood secondary morphological characters and to study genetic variation directly at the molecular level (Cheney and Babbel, 1978). Moreover, it has provided taxonomists with a nearly unlimited number of new stable taxonomic characters.

Protein electrophoresis is only now starting to be applied to problems involving the species and population structure of planktonic algae, about which we know next to nothing.

Planktonic algae are often thought of as freely dispersing homogeneous entities characterized by species-specific physiological parameters (e. g. Lehman et al, 1975). Such a model may not be applicable to all species, as indicated by the mounting evidence for the existence of morphologically indistinguishable ecotypes of many marine diatoms (Guillard and P. Kilham, 1977). Likewise, there is evidence that the distribution of some species is limited by geographical barriers which may be overcome from time to time. The sudden appearance of Melosira binderana in North America in 1961 and its subsequent spread throughout the lower Great Lakes is probably an example of this phenomenon (Round, 1971).

The objective of the present study is to elucidate some aspects of the population and species structure of the common freshwater planktonic diatom, Asterionella formosa Hassal using protein electrophoresis to estimate genetic similarity. More specifically, the objective of the study is to measure the genetic variation within a collection of isolates of the experimental species originating from a variety of habitats and to provide the answers to the following questions: Are populations of the experimental species genetically heterogeneous or clonal? Does the genetic composition of the populations change during the year? Are there

genetic differences among isolates from different populations? Can these differences be correlated with the isolates' geographical origin and ecological characteristics of their habitat? And finally, does the collection consist of discrete groups of genotypes?

LITERATURE REVIEW

1. Biology of A. formosa

1. (a) *Distribution, ecology, and physiology*

A. formosa is a cosmopolitan organism, having been found on all continents including Antarctica (Körner, 1970). It is particularly ubiquitous in temperate lakes and reservoirs where it sometimes produces massive spring and fall blooms, which may impart an unpleasant odour and clog filtration plants (Whipple and Jackson, 1899).

A. formosa is an important component of the phytoplankton of oligotrophic lakes in Western Canada (Rawson, 1956), Labrador (Duthie et al, 1975), Colorado (Koob, 1966), the Soviet Union (Zhadin and Gerd, 1963) and elsewhere, leading some authors to consider this organism to be an indicator of oligotrophy (e. g. Williams, 1969).

On the other hand, A. formosa is often abundant in highly enriched bodies of water (e. g. Lind, 1945; Zhadin and Gerd, 1963; Michalski et al, 1975). Examination of sediment cores from lakes that have become eutrophic in historic times has revealed in several cases that A. formosa frustules are present only in the uppermost strata, indicating that it is a relative newcomer and hence an organism typical of enriched conditions (Round, 1964). Examples of such lakes include Windermere (Pennington, 1943), Esthwaite Water (Round, 1961), and Gull Lake in Michigan (Moss, 1972).

In still other cases the relative abundance of A. formosa is not influenced by the trophic status of its habitat. For example, in Lake Washington sediments it had the same rank before, during, and after a period of eutrophication (Stockner and Benson, 1967).

Early studies of A. formosa in the English Lake District have demonstrated that the large spring maximum in the more enriched lakes is triggered by increasing temperatures and light and terminated by silica depletion

(Lund, 1949; Lund, 1950a; Lund, 1950, Pearsal et al, 1959; Lund et al, 1963), while other factors such as chytrid parasitism are of secondary importance (Canter and Lund, 1948; Canter and Lund, 1951).

Chu (1942) was probably the first person to grow A. formosa in culture, thus opening the way for a large number of physiological studies: Mackereth (1953) and Hughes and Lund (1962) studied the phosphorus metabolism of the species while Talling (1955, 1957a) examined the effect of various environmental factors on photosynthesis and growth. Talling (1957b) found no allelopathy between A. formosa and Fragilaria crotonensis, whereas Jørgensen (1956) showed that A. formosa does not produce an autoinhibitor but may be inhibited by a species of Nitzschia.

More recently, Moed (1973) studied the effects of silica depletion combined with high light intensities and S. S. Kilham (1975) compared the silica uptake kinetics and maximum growth rates of two A. formosa clones. Diel patterns of photosynthesis and phosphate uptake were investigated by Stross and Pemrick (1974) and photoinhibition of photosynthesis by Belay and Fogg (1978). Lund et al (1971) devised a bioassay technique for lakewater using A. formosa as the test organism. This technique was used in a study of Blelham Tarn (Lund et al, 1975) and the Berlin water supply (Bringmann and Kühn, 1971).

Lund (1959), Smayda (1974), and Titman (1975) measured the sinking rate of A. formosa colonies, which is known to quadruple after the culture reaches stationary state (Titman and P. Kilham, 1976). The nutritional state of A. formosa cultures or populations can be identified by the number of cells in each colony; 8 cells per colony are typical of growth at maximum rates, whereas this number drops to two during phosphate limitation and increases to up to twenty during silica limitation (Tilman

et al, 1976).

Tilman (1976) and Tilman and S. S. Kilman (1976) were able to show experimentally that the outcome of competition between A. formosa and Cyclotella meneghiniana is determined by the relative concentrations of phosphate and silica with the former species winning out at high Si/P ratios. This conclusion can be extended to natural A. formosa populations, which seem to thrive at molar Si/P ratios of 500 to 1,000 (Tilman, 1977).

1. (b) *Life history*

The only known stage in the life history of A. formosa is the vegetative one (Lund, 1949). Since there is no resting stage the species cannot survive for long in lake deposits compared to other species (Stockner and Lund, 1970). Likewise, it cannot withstand dessication (Jaworski and Lund, 1970) nor can it survive the passage through the gut of waterfowl (Atkinson, 1972) Consequently it is not clear whether and by what means it disperses over long distances (Round, 1971).

There have been no reports of sexuality or at least of auxospore formation in A. formosa (Lund, 1961; Körner, 1970), despite the fact that it is one of the most studied diatoms. However, the possibility of sexuality in A. formosa should not be excluded because it is present in the closely related araphidate genera Fragilaria (Nipkow, 1953), Synedra (Geitler, 1939), and Diatoma (Tschermak-Woess, 1973).

It has been suggested that the weakly silicified and crooked form of A. formosa known as variety acaroides may have something to do with sexuality; however, it is more likely that variety acaroides is merely a pathological form (Huber-Pestalozzi, 1942; Lund, 1961).

A piece of indirect evidence of sexuality, or at least of asexual

auxospore formation, is the fact that many populations of A. formosa exhibit a cyclic change in frustule length (Wesenberg -Lund, 1908; Nipkow, 1927; Sprenger, 1925). Such cycles usually last several years, are accompanied by much variation in frustule length within each sample (up to 60 μ), and may be complicated by the simultaneous presence of discrete size classes which have been interpreted as different taxa (Lozeron in Hutchinson, 1967; Koob, 1966).

Other populations, such as the one in Lunzer Untersee, are able to divide without a decrease in frustule length (Ruttner, 1937). This population has shown remarkable uniformity in size, showing variation of only 6 μ during the 30 years of its monitoring (Ruttner, 1937).

1. (c) *Classical taxonomy*

The taxonomic treatment of the freshwater members of the genus Asterionella has varied considerably throughout the years.

Whipple and Jackson (1899) in an early review of the biology of the genus recognized but one well-defined freshwater species, namely A. formosa Hassal.

Lozeron (1902; in Körner, 1970) formally described three varieties of this species distinguished solely on the basis of frustule length.

Huber-Pestalozzi (1942) recognized two freshwater species of Asterionella: A. formosa Hassal (proximal capitate end of frustule distinctly larger than distal end in valve view) and A. gracillima (Hantzsch) Heiberg (ends equal in size in valve view). In addition, he recognized two varieties of A. formosa: A. formosa var. acaroides Lemm. and A. formosa var. tatrica Wolosz., the latter reported only from the Tatra Mountains.

Patrick and Reimer (1966) list three species; A. bleakeleyi W. Smith

(a brackish form very similar to A. formosa), A. ralfsii W. Smith var. ralfsii (a dystrophic form with frustule ends even more unequal than A. formosa), and A. formosa Hassal itself. The latter species is in turn divided into two lower taxa; A. formosa Hass. var. formosa and A. formosa var. gracillima (Hantzsch) Grunow.

The entire genus has recently been revised by Körner (1970), employing clonal cultures, field collections, and light and electron microscopy. It was shown by culture studies that the formosa and gracillima forms can be produced by the same Asterionella clone. The author recognizes only A. ralfsii W. Smith (with three newly erected varieties) and A. formosa Hass. as the only two valid freshwater species; listing A. gracillima Heiberg, A. bleakeleyi W.Smith, and A. formosa var. acaroides Lemm. as three of the 36 synonyms of A. formosa Hass.

However, the distinction between the two freshwater Asterionella species recognized by Körner (1970) has not been tested in culture, as no A. ralfsii W. Smith has ever been cultured. Moreover, the North American variety of this species, A. ralfsii var. americana Körner greatly resembles A. formosa. Since there is only a handful of reports of A. ralfsii W. Smith in the literature (Foged, 1962), it is more than likely that this species is not normally distinguished from A. formosa during routine identifications.

1. (d) *Physiological races*

The massive presence of A. formosa in strikingly different habitats has led several investigators to postulate the existence of morphologically indistinguishable ecotypes in this species (Huber-Pestalozzi, 1942; Rawson, 1956; Lund, 1962). However, such suggestions have never been confirmed experimentally.

Allen (1921) found two forms of A. formosa in the San Joaquin River in California that can usually be distinguished by their size. Both forms were present in the river throughout the year, but the shorter one clearly predominated in the cooler months, leading the author to suggest that it is a eurythermal ecotype,whereas the longer form is a warm stenothermal one, favouring temperatures above 20 C.

Ruttner (1937) also described two temperature ecotypes of A. formosa: a cold stenothermal one called variety hypolimnetica found in Alpine lakes at temperatures ranging from 5 to 8 C, and an eurythermal one called variety epilimnetica found at temperatures of up to 16 C in lakes on the Alpine foothills and in the plains of northern Germany. Variety epilimnetica may be abundant in the epilimnion throughout the year, whereas variety hypolimnetica is restricted to the hypolimnion during the summer stratification.

The above two varieties are morphologically indistinguishable except for the length of their frustules, the cold stenotherm being shorter (33-54 μ) than the eurytherm (63-90 μ). The two ecotypes may reproduce in a different manner because populations of the cold stenotherm show almost no variation in length compared to the eurytherm. In at least one lake, Traunsee, the two putative ecotypes occur together.

Samples of A. formosa from warm and cold waters in North America apparently tend to correspond in frustule length to Ruttner's varieties (Körner, 1970). Stankovic (1960) refers the Lake Ohrid population of the species to variety hypolimnetica.

Comparative culture studies of silica uptake half-saturation constants and maximum growth rates involving two isolates of A. formosa from different populations have shown differences in the above parameters;

however, it is not clear how significant these differences are and what portion of the variation within the species they represent (S. S. Kilham, 1975).

Koob (1966) studied the A. formosa populations in two subaplpine lakes in Colorado for two years. He found extreme variation in frustule length, which he interpreted as the presence of five distinct races of characteristic size ranges. Of these five races, only the second smallest was parasitized by the chytrid Rhizophidium.

Recent work with Rhizophidium planktonicum (Canter and Jaworski, 1978, 1979) indicates that A. formosa isolates show strikingly different reactions to a single clone of the parasite. Whereas some isolates produce a hypersensitive reaction and rapid death of nearly all infected cells, other isolates are resistant to the parasite, allowing it to coexist in culture.

2. Physiological races in other algae

Ruttner's (1937) pioneering study of thermal races in planktonic animals and algae concludes that, like A. formosa, there are two thermal races of Synedra acus. However, this assertion was not tested by comparing the growth of the two forms at different temperatures.

More recent studies identify physiological races of algal species on the basis of culture experiments, rather than merely on the basis of their observed ecological or temporal distributions.

Knudsen (1955) found that Tabelleria flocculosa forms a variety of morphologically distinguishable strains in the English Lake district whose characteristics persist in culture. The strains are in some cases typical of individual lakes and drainage basins, but it is not certain whether they are physiologically differentiated from each other.

Several ecotypes have been identified in thermophilic bluegreen algae. Peary and Castenholz (1964) discovered ecotypes with respect to temperature in Synechococcus, and Sheridan (1976, 1979) identified sun and shade ecotypes in a species of Plectonema whose relative abundance showed a distinct seasonal cycle.

Guillard and P. Kilham (1977) list numerous studies that reveal variation in physiological parameters among conspecific isolates of marine diatoms. Such isolates may show differences in vitamin requirements and the ability to use organic carbon sources in the dark (Lewin and Lewin, 1960), temperature and salinity optima and tolerance ranges (Hargraves and Guillard, 1974; Guillard and P. Kilham, 1977), zinc tolerance (Jensen and Rystad, 1974), and nitrate uptake characteristics (Underhill, 1977).

The most extensive evidence of the existence of ecotypes is available for the marine diatom (Thalassiosira pseudonana (formerly Cyclotella nana). A number of clones of this organism have been studied with respect to temperature and salinity requirements and fine morphological differences (Guillard and Ryther, 1962), vitamin requirements (Guillard, 1968), nitrate uptake (Carpenter and Guillard, 1971), silica uptake (Guillard et al., 1973; Nelson et al., 1976), PCB tolerance (Fisher et al., 1973), tolerance to newly synthesized halogenated compounds (Fisher, 1977), and tolerance to complex chemical waste (Murphy and Balastock, 1980).

The above studies reveal the existence of separate neritic and oceanic ecotypes; the latter being more sensitive to herbicides, having narrower salinity and temperature tolerance ranges, and being adapted to life in an oligotrophic environment. The existence of two generically distinct entities has been confirmed by protein electrophoresis (Murphy et. al., 1978)

Several generalizations concerning the nutrient uptake and growth characteristics of different species and, to a limited extent, clones of marine diatoms have been summarized by Guillard and P. Kilham (1977): Oceanic (i.e. oligotrophic) forms have lower K_s values than estaurine (i.e. eutrophic) forms of similar size. Forms with a low K_s for one nutrient tend to have low K_s values for other nutrients. Finally, smaller forms have lower K_s values than larger forms, and also are able to grow faster.

3. Use of Electrophoresis in Algal and Protozoan Systematics

The theoretical basis for the separation of mixtures of soluble proteins by means of electrophoresis and practical instructions for its use have been described many times (e.g. Maurer, 1971; Oelshlegel and Stahmann, 1973). The application of this technique to systematics is introduced by Gottlieb (1971), while Scandalios (1969) and Gooch and Schopf (1970) give instructions for genetic interpretation of isozyme banding patterns.

Electrophoresis, often in conjunction with other methods, has been used with limited success to revise the taxonomy of several algal groups above the species level. Much of the early work is summarized by Holton (1973): Thomas and Brown (1970a) examined 19 species of Chlorococcum and related genera and, in a separate study (1970b), 32 isolates of the genus Protosiphon. Thomas and Delcarpio (1971) compared three species of Chlamydomonas. Several groups of seaweeds were surveyed by Young (1970), Mallery and Richardson (1972), Richardson and Mallery (1973), and Malinowski (1974); whereas Derbyshire and Whitton (1968) and Baker and Holton (1973) worked with groups of bluegreen algae. Thomas and Groover

(1973) examined Chlorosarcinaceae belonging to different genera.

Many of the above studies are merely exploratory in nature, and often are flawed by the fact that only a single isolate of each species was used (e.g. Thomas and Brown, 1970a). On the other hand, the use of electrophoresis as a means of distinguishing between morphologically identical or highly plastic taxonomic entities at or below the species level is providing us with valuable insights into the population and species structure of many algae.

An example of such a study is the work of Schoenberg (1975, 1977) with isolates of the endosymbiotic dinoflagellate Gymnodinium (=Symbiodinium) microadriaticum from different geographical locations and different coelenterate host species. A comparison of the electrophoretic patterns of uniformly cultured isolates indicates that this species consists of a number of morphologically nearly identical ecotypes, each being specific to a host species and possessing a large geographical distribution.

Cheney and Babbel (1978) examined several Florida populations of the red macrophyte Eucheuma isiforme for their electrophoretic banding patterns, and found that all shallow water populations from different localities were highly similar to each other but dissimilar to deep water populations. The conclusion of the authors was that the species consists of two ecotypes with respect to depth.

The work of Murphy et. al. (1978) with isolates of Thalassiosira pseudonana has already been mentioned. It was found that clones from neritic environments are clearly distinguishable from clones isolated from oceanic habitats, whereas a range of intermediate clones can be obtained from the continental slope.

An extensive study of a population of the marine diatom Skeletonema costatum by Gallagher (1977) involved 400 isolates collected over a period of two years. He analyzed the frequency of electrophoretic patterns obtained at different times of the year and concluded that the summer and winter blooms are dominated by genetically different individuals, while both forms were found in pre-bloom populations.

A disturbing aspect of electrophoretic studies of centric marine diatoms was revealed by Murphy (1978), who discovered that freshly isolated clones of T. pseudonana and S. costatum lose their heterozygosity within the first six months of culture due to frequent self-fertilization and auxospore formation. Either allele may become fixed in individual isolates.

Another electrophoretic study (Underhill, 1977) of a marine diatom, Biddulphia sp., involved a comparison of the nitrate uptake parameters and banding patterns of two isolates of this organism. Differences between the two isolates were found in both aspects of the study but the significance of the differences is not clear because of the small number of isolates examined.

Protein electrophoresis has had great impact on the classification of ciliates (see Borden et al., 1977). Several morphologically defined species are known to consist of a number of morphologically indistinguishable sexually isolated species for which Sonneborn (1957) coined the neologism syngen. Tait (1970) found little genetic variation within each of the 14 described syngens of Paramecium aurelia but large differences in electrophoretic banding patterns between different syngens, thus discovering a way to place new isolates into existing syngens without having to resort to laborious mating studies. Likewise, electrophoresis has been used to classify the non-mating strains of Tetrahymena pyriformis (Borden et al.,

1973). In this case, the isolates were found to belong to five groupings, called phenosets by the authors, which do not correspond to previous classifications. Moreover, great confusion in labelling of the experimental strains was found, with supposedly identical isolates received from different laboratories showing significant differences in their banding patterns.

MATERIALS AND METHODS

1. Origin of isolates

The 101 isolates of *A. formosa* that were analyzed by protein electrophoresis came from two sources.

Twelve of them, representing three U.S. populations (Frains L., Baseline L., and L. Michigan), five English populations (Windermere, Rydal W., Esthwaite W., Elterwater, and Rostherne Mere), and two East European populations (L. Ohrid and Goczałkowice Res.); were obtained from other investigators and are listed in Table 1.

Many of these isolates have been employed in previous studies by various authors. The Windermere isolate was used by Titman and P. Kilham (1976) and S.S. Kilham (1975), the latter study also using the Ohrid isolate. The Frains Lake strain was utilized by Titman (1976), Tilman, P. Kilham and S.S. Kilham (1976), Tilman and S.S. Kilham (1976), and by Tilman (1977). Canter and Jaworski (1978) employed the two isolates from Rydal Water, the isolate from Elterwater, and the ones from Esthwaite and Golczałkowice Reservoir. Likewise, the two Rydal Water isolates were used in a later study by Canter and Jaworski (1979), which also involved isolates from Barren L., Caribou L., and Madge L.

The remaining 89 strains, from 22 Canadian and U.S. populations listed in Table 2, were isolated during the course of the present study. Four of these populations (Cataraqui R., Goodspirit L., Mississippi R., and Upper Rock L.) are represented by a single isolate from each and ten (Berens R., Big Portage L., Crooked L., Indian L., Madge L., Red R., St. Mary's R., L. Superior, West Hawk L., and Whirlpool L.) by two or three isolates. The remaining eight populations (L. Manitoba and seven lakes on the Manitoba-

TABLE 1

LIST OF A. FORMOSA POPULATIONS FROM WHICH ISOLATES WERE PROVIDED BY OTHER INVESTIGATORS

NAME	LATITUDE	LONGITUDE	LOCATION	OBTAINED FROM	ORIGINAL DESIGNATION	DATE OF ISOLATION
Lake Michigan	42°05'W	86°37'W	Benton Harbor, Michigan	S.S. Killam	LM Af A x 1	Fall '75
Frains Lake	42°20'N	83°37'W	Ann Arbor, Michigan	S.S. Kilham	Fra Af	Fall '74
Baseline Lake	42°25'N	83°53'W	Pinckney, Michigan	S.S. Kilham	BL Af D3	Summer'75
L. Ohrid	41°00'N	20°45'E	Ohrid, Macedonia	S.S. Kilham	AfOH2	June '73
Windermere	54°22'N	02°56'W	English Lake Dist.	S.S. Kilham*	L262	?
Rydal Water	54°28'N	02°59'W	English Lake Dist.	H. M. Canter & G.H.M. Jaworski	L277	Oct. '75
Rydal Water	54°27'N	03°00'W	English Lake Dist.	H. M. Canter & G.H.M. Jaworski	L292	Oct. '76
Esthwaite W.	54°21'N	02°59'W	English Lake Dist.	H. M. Canter & G.H.M. Jaworski	L281	1976
Esthwaite W.	54°21'N	02°59'W	English Lake Dist.	H. M. Canter & G.H.M. Jaworski	L302	March '77
Elterwater	54°26'N	03°02'W	English Lake Dist.	H. M. Canter & G.H.M. Jaworski	L297	1976
Rostherne M.	53°21'N	02°24'W	Manchester, England	H. M. Canter & G.H.M. Jaworski	L301	1977
Goczałkowice Reservoir	49°56'N	18°50'E	Bielsko-Biała, Poland	H. M. Canter G.H.M. Jaworski	L265	August 1973

*Isolated by J.W.G. Lund.

TABLE 2

LIST OF *A. FORMOSA* POPULATIONS FROM WHICH ISOLATES WERE OBTAINED DURING PRESENT STUDY

NAME	LATITUDE	LONGITUDE	LOCATION	DATE OF ISOLATION	NUMBER OF ISOLATES
BARREN L.	49°43'N	95°16'N	Whiteshell Prov. Park, Manitoba	Oct. 11, 1976	8
BARREN L.				May 8, 1977	1
BERENS R.	52°20'N	97°00'W	Berens River, Manitoba	June, 1977	2
BIG PORTAGE L.	42°25'N	83°55'W	Pinckney, Michigan	June 24, 1977	2
CARIBOU L.	49°44'N	94°54'W	Kenora, Ontario	Oct. 11, 1976	3
CARIBOU L.				May 8, 1977	2
CATARAQUI R.	44°18'N	76°26'W	Kingston Mills, Ontario	May 12, 1976	1
CROOKED L.	52°20'N	83°58'W	Pinckney, Michigan	June 24, 1977	3
FALCON L.	49°42'N	95°16'W	Whiteshell Prov. Park, Manitoba	Oct. 11, 1976	2
FALCON L.				May 8, 1977	3
GOOD SPIRIT L.	51°31'N	102°40'W	Yorkton, Saskatchewan	July 16, 1977	1
GRANITE L.	49°43'N	94°53'W	Kenora, Ontario	Oct. 11, 1976	4
GRANITE L.				May 8, 1977	1

Continued ...

TABLE 2: LIST OF A. FORMOSA POPULATIONS FROM WHICH ISOLATES WERE OBTAINED DURING PRESENT STUDY (Continued)

NAME	LATITUDE	LONGITUDE	LOCATION	DATE OF ISOLATION	NUMBER OF ISOLATES
INDIAN L.	44°35'N	76°19'W	Chaffey's Locks, Ontario	May 12, 1976	2
L. OF THE WOODS	49°46'N	94°30'W	Kenora, Ontario	May 30, 1976	3
L. OF THE WOODS				May 8, 1977	2
LYONS L.	49°43'N	95°11'W	Kenora, Ontario	October 11, 1976	11
MADGE L.	51°40'N	101°38'W	Duck Mtn. Prov. Park, Sask.	May 9, 1977	3
L. MANITOBA	50°11'N	98°24'W	Delta, Manitoba	May 23, 1976	3
L. MANITOBA				Nov. 5, 1976	7
L. MANITOBA				May 14, 1977	5
MISSISSIPPI R.	47°14'N	93°30'W	Cohassett, Minnesota	May 1, 1976	1
MOTH L.	49°44'N	95°00'W	Kenora, Ontario	May 8, 1977	4
MOTH L.				June 9, 1977	2
RED R.	49°49'N	97°06'W	Fort Garry, Manitoba	April 8, 1977	3
ST. MARY'S RIVER	46°30'N	84°21'W	Sault Ste. Marie, Ont./Mich.	June 26, 1977	2
L. SUPERIOR	48°50'N	87°43'W	Rossport, Ontario	June 26, 1977	2
UPPER ROCK L.	44°30'N	76°24'W	Chaffey's Locks, Ontario	May 12, 1976	1
WEST HAWK L.	49°44'N	95°12'W	Whiteshell Prov. Park, Manitoba	June 9, 1977	2
WHIRLPOOL L.	50°43'N	99°48'W	Riding Mtn. Nat. Park, Manitoba	June 5, 1977	3

Ontario border) are represented by 5-15 isolates from each lake obtained either simultaneously, at different times of the year, or in consecutive years.

A. formosa was not isolated from (or detected in) all of the lakes sampled. Many of the sampled lakes did not have any A. formosa, while many isolated colonies failed to grow in the laboratory. Moreover, for various reasons (e.g., excessive dilution, chytrid infection, accidents) many isolates became extinct before their analysis by protein electrophoresis was possible.

2. Limnology of experimental lakes and rivers

Some of the basic limnological characteristics of the 32 bodies of water from which A. formosa isolates were obtained are summarized in Table 3, while additional information is presented in the subsequent text. This information has been gleaned from the literature and from analysis of water and plankton samples. More extensive information is available for Lake Manitoba and the eight lakes on the Manitoba-Ontario border, which were sampled on a monthly basis throughout the 1977 ice-free season. The total phosphorus concentration, which is a good indicator of trophic status (Carlson, 1977), for several of the lakes was determined in May, 1977 by Mr. M. Chapel of the Analytical Chemistry Unit, Freshwater Institute, Winnipeg.

Windermere, Esthwaite Water, Elterwater, and Rydal Water (Lund, 1949; Macan, 1970) These lakes comprise a part of the English Lake District, which was formed during deglaciation some 9000 y.b.p. Windermere is the largest of the four lakes and receives the waters of the latter three.

TABLE 3

SOME LIMNOLOGICAL CHARACTERISTICS OF EXPERIMENTAL LAKES AND RIVERS

NAME	pH	CONDUCTIVITY μ mho/cm	TOTAL ALKALINITY mg/l	HCO_3^- mg/l	$CO_3^=$ mg/l	Na^+ mg/l	K^+ mg/l	Mg^{++} mg/l	Ca^{++} mg/l	Cl^- mg/l	$SO_4^=$ mg/l	TOTAL P μg/l	SECCHI DEPTH m
BARREN L.	6.9-7.5*	48*	19*									29*	0.5-2.5*
BERENS R.	6.9-7.2*	75*		23	0	3	1	2	6	2	3		
BIG PORTAGE L.	8.3*	320*											
CARIBOU L.	6.9-7.1*	65*	16*									20*	1.5-2.0*
CATARAQUI R.	7.8-8.5	200	73-105							4	17	17-76	
CROOKED L.	8.0*	320*	160*										2.8*
FALCON L.	6.8-7.8*	98*	50*									14*	2.0-4.5*
GOODSPIRIT L.	8.6*	960*	220*										0.5*
GRANITE L.	7.0-7.5*	63*	18*									18*	3.0-3.5*
INDIAN L. **	7.7	215	75-100	113	0	3	12	6	48	8			
L. OF THE WOODS	7.6-8.2*	111*	54*	53-64	0	2	1	4	14	2	8	17*	2.0-3.5*
LYONS L.	7.2-8.1*	155*	87*									23*	2.5-3.0*
MADGE L.	8.3-8.5*	725*	144*	185	0	11	10	44	57	2	184	65*	1.5-2.3
MANITOBA	8.1-8.6	2390*	240	318	24	246	19	95	47	532	218	36*	1.6
MISSISSIPPI R.	7.7	295	135			4		13		3.5	21	50	
MOTH L.	6.8-7.5*	90*	35*									21*	1.5-3.5*
RED R.	7.8-8.0*	1132	200-300	145	0	13	4	16	36	6	50	116*	0.5*
ST. MARY'S R.	7.3-8.0	75-100		52	0	1	1	3	14	1.5	5		4.8*
SUPERIOR	7.5-8.0	95*	44	48	0	1	1	3	13	1.5	3	10	6.0-12.6
UPPER ROCK L.		100-150	75-100										
WESTHAWK L.	7.0-7.7*	65*	20*									6*	6.0-9.5*
WHIRLPOOL L.	8.6	232	143	129	14	1	1	14	14	2.5	0	49	
MICHIGAN	8.5	261	109						37	7	15	13	4.8
FRAINS L.													
BASELINE L.													
OHRID	8.0-8.9		84	133				9	29	4	8		16
GOCZAŁKOWICE RES.	7.0		27-108					5	28	14	23		0.75-2.0
WINDERMERE				10		4	1	1	6	7	7		
RYDAL W.													
ESTHWAITE W.				18		5	1	3	8	8	10		3.1
ELTERWATER													
ROSTHERNE MERE			41						27	32		64	

* Data obtained during present study. Other data are averages from references listed in text.

** Data for Clear Lake (directly downstream of Indian Lake).

Together with Esthwaite Water it is the most productive and has a larger A. formosa population, which forms a large spring bloom and a smaller fall bloom. In late summer, the plankton is dominated by bluegreen algae.

Rostherne Mere (Lind, 1945) is a highly calcareous and productive Cheshire lake; A. formosa occurs here throughout the year, accounting for up to 89% of the phytoplankton. It has increased in abundance since the turn of the century and is characterized by larger frustules (50-82 µ) than in neighbouring lakes.

Goczałkowice Reservoir (Bombówna, 1962) in Poland was formed in 1955 by damming the Wisla River. It is an eutrophic reservoir, and A. formosa is found here throughout the year. It forms large spring and fall maxima.

Lake Ohrid (Stankovic, 1960; Allen and Ocevski, 1976) is shared by Yugoslavia and Albania. It is a spring-fed marl lake of extreme depth (298 m) and oligotrophy. It occupies a graben of Middle Pliocene age, thus being about three million years old. According to Antevs (1929) it is the only lake of the ones examined in this study that was not covered by ice during the maximum advance of the glacier during the last Ice Age. It is known to contain numerous endemic plankton species including nine algal ones. A. formosa (variety "hypolimnetica"), Stephanodiscus astraea, and two endemic Cyclotella spp. are the dominant phytoplankters, while some common diatoms are missing.

L. Michigan, L. Superior, and St. Mary's River (Thomas and Gale, 1965; Williams and Scott, 1962; Schelske and Roth, 1973). Lake Superior is a large oliogotrophic lake with dilute water. In June 1977, the plankton near Rossport was dominated by various diatoms, Dinobryon,

and Ceratium hirundinella. A. formosa (frustule size 47-85μ ; almost all of the "acaroides" form), the net plankton of Big Portage Lake consisted almost entirely of this species (frustule size 63-99μ; "acaroides" form rare). Otherwise the plankton of the two lakes was similar, with blue-greens (Lyngbya, Oscillatoria), greens (Pediastrum, Scenedesmus) and diatoms being common.

Cataraqui R., Indian L., Upper Rock L. (Swabey, 1953; Ryder, 1964; Sparling and Nalewajko, 1970; Ont. Min. of the Environment, 1972, 1974). All of the Southeastern Ontario sites are within the Cataraqui River watershed. Indian Lake and Upper Rock Lake are surrounded by an extension of the Canadian Shield known as the Frontenac Axis, and are therefore relatively dilute and mesotrophic. Both experience A. formosa peaks in the spring and summer, but in the summer only small populations of this species remain in the hypolimnion. The Cataraqui River site is located below an unnamed highly eutrophic reservoir, which experiences heavy cyanophyte blooms throughout the summer. A. formosa is present here only in the spring and fall.

Mississippi R. (Dr. P. Lee, personal communication). The upper Mississippi River in Minnesota is a mesotrophic body of water. Only a very small A. formosa population was found there in May, 1976.

Red River (Thomas, 1959). This river has a high nutrient content, from both natural and cultural causes. Due to its high silt content it supports small algal populations. Only a few colonies of A. formosa (frustule size 68-87μ) containing many dead cells were found in April, 1977; other algae present being Melosira spp, greens, bluegreens and numerous benthic diatoms. The water contains a high concentration of

dissolved salts, whose amounts vary greatly with the flow.

Goodspirit Lake and Madge Lake (Kuehne, 1941; Thomas, 1959; Rawson and Moore, 1964) are mildly saline Saskatchewan lakes occupying shallow basins with no outlets. The halophilic diatom Chaetoceros elmorei is present in both of them. Madge L. was sampled in May and July 1977 and on both occasions a healthy population of A. formosa was found (frustule 59-99 μ; "acaroides" form present). The phytoplankton consisted of miscellaneous diatoms, greens, and bluegreens. Goodspirit Lake resembles Madge Lake physically; it also has a shallow basin and consequently does not stratify. In June 1977, it had only a negligible A. formosa population (frustule size 59-75 μ; "acaroides" form present) with many dead cells. Its phytoplankton was dominated by bluegreen and green algae.

Whirlpool Lake (Parks Canada, unpublished) is a shallow (1.7 m) calcareous lake within the Riding Mountain National Park. In July 1973 the plankton consisted mostly of bluegreen and green algae, and in June 1977 it contained almost no A. formosa (frustule length about 54 μ). According to Ritchie (1967) the Riding Mountain area formed the Western shoreline of Glacial Lake Agassiz.

Lake Manitoba (Thomas, 1959; Cober, 1968) is a large (4 600 km^2) but shallow (max. depth 7 m) remnant of Glacial Lake Agassiz characterized by relatively saline water, much silt, and no summer stratification. A. formosa is not known to form large populations in this lake. Small populations (frustule size 45-84 μ) containing many dead cells were found at its Southern tip at Delta in May and September 1977 and in November 1975 but not in the summer. The plankton consists chiefly of diatoms,

bluegreens, and greens.

Berens River (Thomas, 1959) is a large brownwater river draining a portion of the Canadian Shield on the Eastern shore of Lake Winnipeg. Only a few colonies of A. formosa (frustule size 56-96 μ) were found in mid-June 1977, the plankton being dominated by Tabellaria, Fragilaria, Dinobryon and desmids.

Barren L., Caribou L., Falcon L., Granite L., Lyons L., Moth L., West Hawk L., and Lake of the Woods (Bajkov, 1933; McLeod, 1943; Lowe, 1924; Thomas, 1959). The first seven of these lakes are small dilute Canadian Shield lakes along a 40 km stretch of the Trans-Canada Highway on the Manitoba-Ontario border in the Winnipeg River drainage basin. They are mesotrophic with the exception of the deep (101 m) and oligotrophic West Hawk Lake. The lakes occupy a height of land; Moth L. and Lyons L. drain north via other lakes into West Hawk L., whereas the rest drain south. Caribou L. drains via Granite L. to L. of the Woods, while Barren L. drains to Falcon L. and then also to Lake of the Woods. Barren L. is a brownwater lake with abundant A. formosa population (frustule size 61-110 μ) in the spring and fall; however, it disappears in the summer and is replaced by a succession of cyanophyte blooms (Gomphospheria, Oscillatoria). Caribou Lake is also a brownwater lake containing an abundant and large-celled A. formosa population (frustule size 56-120 μ) that remains throughout the summer, when the phytoplanktons is comprized of desmids, diatoms, and a mild Microcystis aeruginosa bloom. In Falcon Lake A. formosa (frustule size 63-93 μ) also remains abundant throughout the summer. In Granite Lake, A. formosa (frustule size 49-110 μ) was present throughout the summer, simultaneously with an early summer Aphanizomenon flos-aquae bloom.

The Lyons Lake A. formosa population is small-celled (frustule size 45-85 μ) and disappears in the summer. Its place was taken by an Anabaena flos-aquae bloom. Moth Lake is also a brownwater lake with plankton dominated by desmids, Tabellaria, Cyclotella, and Dinobryon. In the summer it experienced a Gloeotrichia bloom. Its A. formosa population (frustule range 56-85 μ) was abundant throughout the summer and reached a maximum of 1.8×10^6 cells/l in the fall, which is the highest concentration recorded in this study. In West Hawk Lake A. formosa (frustule size 54-99 μ) did not appear until June and continued to be present until November, the phytoplankton consisting largely of diatoms. Lake of the Woods is several thousands km^2 in size and consists of many basins. Its phytoplankton sampled at Rat Portage Bay contained A. formosa (frustule size 47-96 μ; "acaroides" form present in May) throughout the summer. It was dominated by bluegreens, greens, and diatoms; and in September 1976 it experienced a heavy Aphanizomenon flos-aquae bloom. The drainage basins of the above eight lakes were occupied until about 8 300 y.b.p. by Glacial Lake Agassiz (Elson, 1967).

3. Isolation procedure

Net plankton samples from each body of water were obtained by repeatedly towing a Wisconsin plankton net over a distance of 4 m. Each sample was placed in a labelled tightly capped 30 ml culture tube and kept in an ice bucket until transported into the laboratory, where it was scanned for A. formosa colonies under a dissecting microscope at 40 x magnification.

Colonies that were obviously free of chytrid parasites were picked out with a Pasteur pipette, the tip of which had been drawn out to form a fine capillary tube. They were then separated from other algae by repeated transfers into drops of sterile distilled water. Finally, individual colonies were micropipetted into polystyrene petri dishes (10x35 mm) containing sterile Woods Hole MBL medium, whose composition is given in Appendix I. The petri dish cultures, allowing unimpeded observation of growth under a dissecting microscope, were kept in a growth cabinet at 10-15 C and 300-500 lux of continuous white light.

After two to three weeks, each petri dish culture was checked for the characteristic brown colour and coarse appearance of A. formosa colonies, while cultures contaminated with chytrids or microscopic algae were purified using the above isolation procedure.

4. Maintenance of culture collection

Isolates of A. formosa showing good growth in petri dish culture were transferred into 30 ml glass culture tubes with rubber lined screw caps containing 20 ml of Woods Hole MBL medium (see Appendix I). These were maintained in horizontal position at about 5 C and in low light (100 lux).

The above arrangement eliminated the twin problems of evaporation and contamination. Nevertheless, the stock cultures were regularly checked for green colour and clumping, which indicate respectively algal and chytrid contaminants, using a dissection microscope.

Since the stationary-phase stock cultures experienced a gradual die-off and sticking to the glass walls in addition to an increase in the number of bacteria, they were rejuvenated at approximately monthly intervals. This procedure consisted of vigorously shaking each culture tube, decanting roughly three-quarters of the volume of the culture, and replacing it with fresh sterile Woods Hole MBL medium.

5. Batch cultures

Batch cultures were initiated by pouring about a half of the volume of a stock culture into a 1800 ml Erlenmeyer flask containing 500 ml of freshly prepared sterile Woods Hole MBL medium (see Appendix I) and capped with a plastic foam stopper.

Three to four days later, after making certain that the culture was not killed during the transfer, the inoculum was further diluted to a 4 000 ml and distributed among four 1 800 ml Erlenmeyer flasks. The batch cultures then were allowed to grow for four to six days in a growth cabinet at 15 - 17 C and 1100 lux of continuous white light, provided by cool white Sylvania fluorescent tubes. All Erlenmeyer flasks were manually shaken at least once a day.

6. Harvest and protein extraction

As soon as the batch culture reached stationary phase, indicated by a rapid increase in the settling rate of the A. formosa colonies, it was harvested by filtering the contents of the 1800 ml Erlenmeyer flasks through #10 Nitex nylon bolting cloth. This cloth possesses diagonal apertures of 10-15 μ and thus retain practically all A. formosa colonies but passes bacteria.

A. formosa cells were then washed off the nylon sieve, resuspended in 10 ml of distilled water, and sedimented in a general laboratory centrifuge at low speed (1 800 x g) for five minutes. The supernatant was discarded, while the pellet was resuspended in 10 ml of cold homogenation buffer (pH= 6.7), the composition of which is given in Appendix II.

The resuspended pellet was broken up in a sonicator/homogenizer (Kinematica GmbH, Switzerland) for three one minute intervals at Speed seven, alternated with one minute ice baths in order to keep the temperature of the extract below 15 C.

The homogenized extract was then sedimented using a refrigerated high speed centrifuge at 12 000 x g at 1-5 C for ten minutes. The pellet, containing broken frustules and cell fragments, was discarded, whereas the yellowgreen supernatant (i.e. the crude protein extract) was kept in an ice bucket until electrophoresis. The protein yield was 125-160 µg/ml crude extract, as determined by the method of Lowry et al. (1951)

7. Gel preparation

The discontinuous gel system of Davis (1964), listed in Maurer (1971) as System #1a, was used. In this system the gels are enclosed in transparent plastic tubes during electrophoresis and consist of two parts: a short dilute stacking gel with pH= 6.7 that concentrates the protein sample and a longer 7.5% separation gel with pH= 8.9.

The 10 cm gel tubes were permanently marked at the 2 cm and 4 cm level to simplify the gel preparation, since the uppermost 2 cm (0.35 ml) of each tube contained the protein sample, the next 2 cm contained the stacking gel, while the remaining 6 cm (= 1.15 ml) of the tube was taken up by the separation gel.

All gels were made in batches of twelve less than an hour prior to their use. The required monomer (acrylamide), cross-linking agent (Bis), and catalysts (TEMED) and ammonium persulfate) were obtained from BioRad Laboratories, whereas another catalyst (riboflavin) and TRIS were from Sigma. These materials were used to make 100 ml batches of six stock solutions, the composition of which is listed in Appendix II. The stock solutions were stored at 5 C in dark bottles, and were allowed to warm up to room temperature prior to use.

Clean gel tubes were placed on a rack and their lower ends were closed with rubber caps. The separation gel polymerizing solution was made first by mixing stock solutions I, II and III in the ratio 1:1:2 for a total of 14 ml. The lowermost 6 cm of each gel tube was then filled and the meniscus on top of the separation gel solution was flattened by gently introducing a few drops of distilled water by a microsyringe, taking great care not to mix the water with the more dense polymerizing solution.

The separation gel polymerized within 15 to 20 minutes and the water layer was gently removed with a micropipette. It was replaced with 2 cm of unpolymerized stacking gel solution, which had been prepared by mixing stock solutions IV, V, and VI in that order in ratios of 2:1:1 for a total of 4 ml. The meniscus was again flattened by a few drops of water introduced by a microsyringe. Finally, the stacking gel was allowed to polymerize under a fluorescent light, which normally took 30 minutes.

8. Electrophoresis

Completion of polymerization of the stacking gels was identified by formation of a visible interface between the gel and the overlying water. The water layer was withdrawn with a micropipette, the rubber caps were removed from the lower ends of the gel tubes, and the tubes themselves were

inserted into numbered slots in the chamber of the electrophoretic apparatus (Buchler Instruments).

The upper and lower reservoirs of the apparatus were filled with 5 C electrode buffer of pH = 8.3, the composition of which is given in Appendix II. The stock buffer was diluted tenfold with 5 C distilled water immediately prior to use.

Thirty µl of a tracking dye solution (0.01% bromphenol blue in 01. N NaOH) and 100 µl of 40% sucrose solution were added to each ml of the protein sample dissolved in the homogenation buffer. Approximately 350 µl of the protein sample, equivalent to 45-55 µg protein, was gently layered onto each gel with a micropipette. Air bubbles, which may interfere with electric current flow, were removed and the lid of the apparatus placed in position. The cooling jacket was connected to a cold water tap and the electrodes connected such that the upper reservoir was negative.

A Vokam power source (Shandon Southern Co.) provided a steady current of 1.0 mA per gel (total current between reservoirs= 12 mA). As soon as the protein sample, migrating downwards, became concentrated as s single band at the stacking-separation gel interface, the current was increased to 3.0 mA per gel.

Each electrophoresis run took 110-135 minutes. The current was stopped as soon as the tracking dye band approached within 0.5 cm of the lower end of the separation gel. The reservoir buffer was discarded and the gel tubes removed from the apparatus.

9. Protein staining and enzyme assays

Immediately after the end of electrophoresis the gels were expelled from the tubes by means of a small syringe piston and rinsed with distilled water.

Each batch of twelve gels consisted of four gels from three isolates. One gel of each isolate was stained for GP, while the other three were assayed for EST, AcP, and MDH activity. A preliminary study, often using samples from mixtures of isolates, included assays for AlP, GDH, and LAP activity. However, these three enzymes showed a single band of activity at constant R_f values, indicating monomorphic loci, and were consequently excluded from further study.

Gels to be assayed for EST, MDH, and AcP activity were cut at the level of the tracking dye band in order to mark its location. They were then placed in glass culture tubes containing 20 ml of freshly prepared assay mixtures, the composition of which is given in Appendix III. Bands indicating enzyme activity were allowed to develop at room temperature overnight.

Gels to be stained for non-specific protein (=GP) were not cut because a protein fraction with mobility identical to that of the tracking dye was present in all extracts. The gels were fixed for 30 minutes in 12% Trichloroacetic acid solution, which denatures and thus immobilizes all protein fractions, and stained for one hour with a Coomassie Brilliant Blue R-250 (Eastman Kodak Co.) solution (0.1% in 7% acetic acid). Since this dye stains not only proteins but also the gel itself, the gels were destained in 7% acetic acid for 24 hours. The destaining solution had to be changed frequently.

The gels were rinsed in distilled water and stored in labelled 30 ml culture tubes containing 7% acetic acid.

Finally, bands of GP and enzyme activity were scored within days or weeks of electrophoresis by placing each gel onto back-illuminated graph paper with mm divisions and measuring the position of the centre of each band and of the tracking dye band from the stacking-separation gel interface. The R_f value, representing the relative mobility of each band with respect to the tracking dye band as a percentage, was calculated for each band on each gel.

10. Tests of validity of electrophoretic data

Three aspects of the validity of the experimental procedure and the resulting data (Ressler, 1973) were confirmed during a preliminary investigation: their reproducibility, the enzymatic nature of putative enzyme activity bands, and the lack of an effect of bacteria associated with cultures of the experimental species on the electrophoretic banding patterns.

Reproducibility of the electrophoretic banding patterns was found to be controlled by the quality of the gels and to a lesser extent, by the soluble protein concentration in each crude extract. While the former influences the relative electrophoretic mobility of all proteins, the effect of the latter is restricted largely to varying the number of detectable GP bands. Since different A. formosa isolates were found to have widely different growth rates in batch culture, standardization of the protein concentration in crude extracts was achieved by harvesting the batch cultures after they have reach stationary phase, rather than harvesting them at a fixed chronological age. Uneven gel quality is to some extent inherent to the technique of disc electrophoresis because each gel polymerizes separately, unlike slab electrophoresis where multiple samples are run side-by-side on the same gel (Oelshlegel and Stahmann, 1973).

The R_f values of individual protein bands, obtained weeks and months apart with different crude protein extracts of the same isolate and with different batches of gels, were found to be reproducible to within 1.5%. The comparable statistic is rarely listed in the literature, but is known to vary from 0.1% (Shechter et al., 1968) to 3 - 8% (Grant and Proctor, 1980).

The enzymatic nature of putative enzyme activity bands was tested by attempting to assay gels that had been boiled for a few minutes and by running enzyme assays without the substates. With the exception of EST and to some extent AlP, no bands appeared when the enzyme had been denatured or the substrate missing.

In the case of EST and AlP several faint nonenzymatic bands appeared, with Rf values identical to the major GP bands, indicating binding of the Fast Blue RR salt directly to protein. Fortunately, it was possible to distinguish such bands from enzymatic ones by their colour.

A test for possible effects of bacterial enzymes on the electrophoretic banding patterns was carried out by electrophoresing a centrifuged and homogenized Nitex bolting cloth filtrate of A. formosa batch cultures. However, neither GP nor enzyme activity bands were found, indicating that the number of bacteria involved was too small to have an effect.

11. Statistical treatment of data

Lists of R_f values of all isolates were converted to binary absence/presence data, considering those bands whose R_f values were within 1.5% to be homologous.

The degree of association of different electrophoretic banding patterns was studied using cluster analysis, a numerical taxonomy technique

for objective sorting of individuals into groups. This technique is particularly appropriate when little or nothing is known about the category structure of the data (Wishart, 1978).

The hierarchical fusion technique, a variant of cluster analysis devised by Ward (1963) and Orloci (1967), was used. In this technique, each cluster of one or more individuals is described by its centroid and by its error sum of squares, the latter defined as the sum of the Euclidean distances from each individual to the centroid of its parent cluster. A tight cluster, consisting of highly similar individuals, will have a low error sum of squares compared to a loose cluster.

The first computational cycle starts with each individual being considered as a separate cluster and involves the calculation of the increase in the total error sum of squares which would accompany the fusion of each possible pair of clusters (i.e. individuals). The fusion of the two most similar clusters, i.e. that which yields the least increase in the total error sum of squares is selected (Wishart, 1978). Succeeding computational cycles decrease the number of clusters through fusion until all individuals are included in a single cluster.

Since cluster analysis of individuals with a large number of characters involves much repetitive computation, the operation was computerized using the CLUSTAN package (Version 1C, release 2) available through the University of Manitoba Computer Centre. This program is based on an algorithm for cluster analysis using a dissimilarity matrix (Wishart, 1969) and its use is described by its originator (Wishart, 1978). It is also capable of plotting the results of cluster analysis in the form of a dendrogram.

RESULTS

Multiple isolates from the same population did not show electrophoretically detectable differences from each other. This was true for all of the twenty populations from which more than one isolate were obtained, including the seven populations from which clones were obtained at different times of the year or in different years.

Isolates from different populations, i.e. from different bodies of water, showed differences in their electrophoretic banding patterns of 3 to 28 bands out of 41 corresponding to dissimilarity coefficients ranging from 0.073 to 0.683. The electrophoretic banding patterns characteristic of each population are presented in Figure 1.

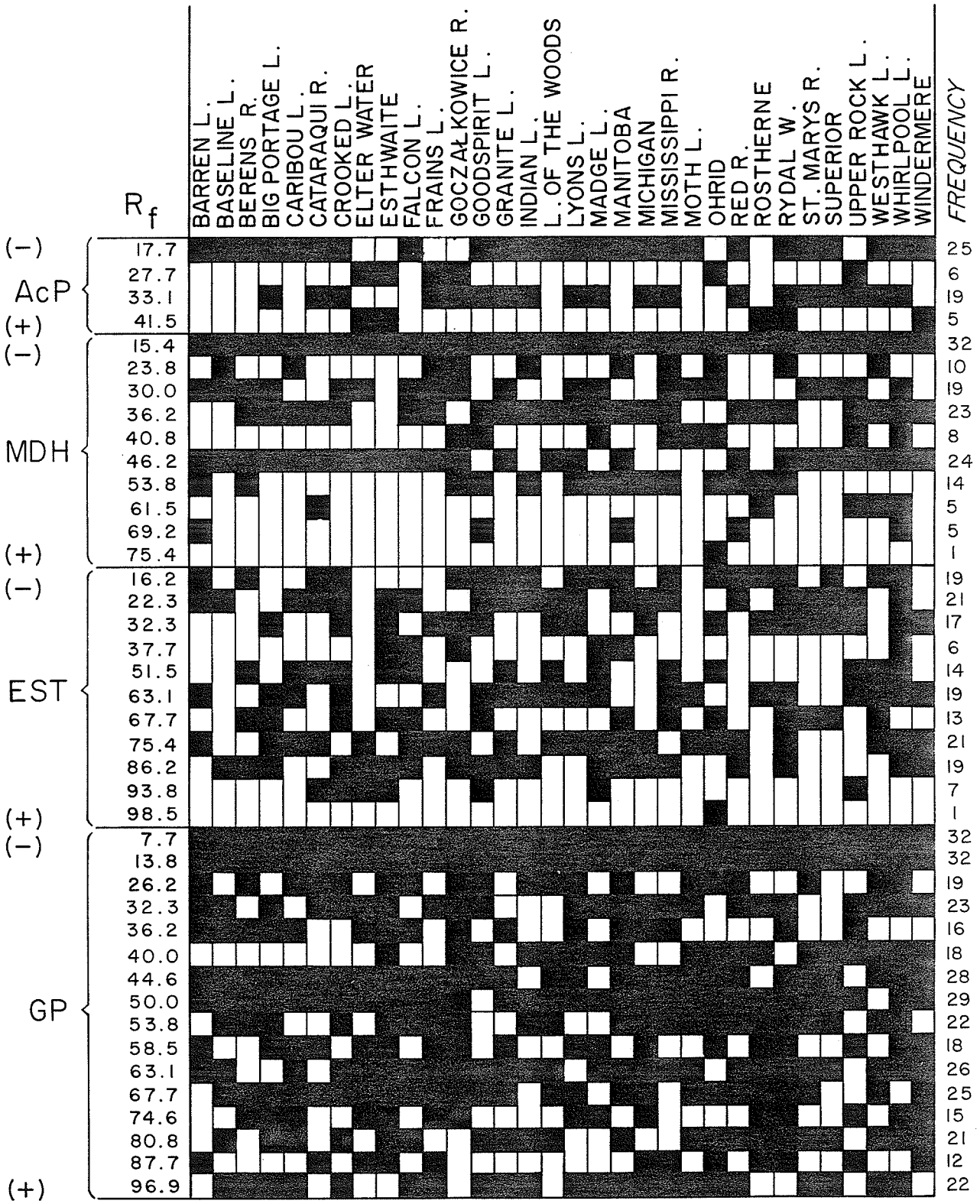
Four different AcP bands were scored in the collection of isolates, with two of them confined almost exclusively to the European isolates. Each isolate possessed one to three bands.

A total of ten different MDH fractions were scored, of which one is shared by all the isolates whereas another one is unique to the Lake Ohrid isolate. The number of MDH bands exhibited by individual isolates varies from two to seven.

Eleven EST bands were scored, none of them being common to all of the isolates. Again, the Lake Ohrid isolate possesses a private band of fast electrophoretic mobility. The number of EST bands per isolate ranged from two to eight.

Sixteen different GP fractions were scored. Some of them had identical Rf values with certain enzyme activity bands, indicating possible homology. The two slowest GP fractions were present in all but a handful of isolates. No GP bands were found to be unique to a particular population, and the number of GP bands was 9 - 13 per isolate.

FIG. 1. DIAGRAMMATIC REPRESENTATION OF POPULATION-SPECIFIC ELECTROPHORETIC BANDING PATTERNS.



A total of 41 GP and enzyme activity bands were scored. The total number of bands possessed by individual isolates ranged from 18 in the case of the Baseline Lake population to 30 (Esthwaite Water).

The degrees of dissimilarity between members of each of the 496 possible pairs of population-specific electrophoretic banding patterns can be ascertained from the dissimilarity matrix presented in Table 4. This matrix lists the dissimilarity coefficient for each pair, defined as the number of unshared bands, in addition to the average dissimilarity coefficient value for each population. This statistic varies from 0.320 to 0.476 and is an indicator of the uniqueness of each population--specific electrophoretic banding pattern. It is not surprising that the Lake Ohrid isolate has the highest value, followed by Upper Rock Lake, Esthwaite, Elterwater, and Rostherne Mere.

The frequency distribution of dissimilarity coefficient values, presented in Figure 2, shows a single mode characteristic of continuous variation of characters rather than the existence of discrete clusters of genetically similar isolates (Borden et al., 1973)

The results of cluster analysis appear in the form of a dendrogram in Figure 3. The plotted coefficient value is equal to twice the increase in the error sum of squares caused by fusion, and the total error sum of squares for any clustering stage can be obtained on division by two of the cumulative sum of the values which precede that clustering stage in the clustering sequence (Wishart, 1978).

The dendrogram indicates that the first populations to form clusters are Lake Superior and St. Mary's River followed by Lake Manitoba and Red River and again, by Caribou Lake and Lake of the Woods. In all three cases the members of each pair of populations are limnologically similar and geographically close. The last individual populations to join clusters are

TABLE 4

DISSIMILARITY MATRIX FOR 496 POSSIBLE PAIRS OF POPULATION - SPECIFIC ELECTROPHORETIC BANDING PATTERNS (COEFFICIENT OF DISSIMILARITY = (NUMBER OF UNSHARED BANDS)/(TOTAL NUMBER OF BANDS))

	BARREN L.	BERENS R.	BIG PORTAGE	CARIBOU L.	CATARAQUI	CROOKED L.	FALCON L.	GOOD SPIRIT	GRANITE L.	INDIAN L.	L. OF WOODS	LYONS L.	MADGE L.	MANITOBA	MISSISSIPPI	MOTH L.	RED R.	ST. MARY'S	SUPERIOR	UPPER ROCK	WESTMAN L.	WHIRLPOOL L.	MICHIGAN	FRAINS L.	BASELINE L.	OHRID	GOCZAKOWICE	WINDERMERE	RYDAL W.	ESTHWAITE W.	ELTERWATER	ROSTHERNE M.
BARREN L.	0																															
BERENS R.	0.390	0																														
BIG PORTAGE L.	0.415	0.268	0																													
CARIBOU L.	0.317	0.317	0.293	0																												
CATARAQUI R.	0.293	0.390	0.415	0.317	0																											
CROOKED L.	0.415	0.268	0.244	0.341	0.317	0																										
FALCON L.	0.390	0.195	0.268	0.220	0.390	0.220	0																									
GOOD SPIRIT L.	0.439	0.439	0.415	0.463	0.439	0.220	0.439	0																								
GRANITE L.	0.268	0.317	0.244	0.195	0.268	0.244	0.220	0.415	0																							
INDIAN L.	0.415	0.366	0.439	0.341	0.463	0.293	0.366	0.268	0.341	0																						
L. OF WOODS	0.390	0.341	0.317	0.171	0.341	0.220	0.195	0.341	0.268	0.268	0																					
LYONS L.	0.244	0.293	0.220	0.268	0.293	0.317	0.390	0.341	0.268	0.366	0.293	0																				
MADGE L.	0.415	0.415	0.390	0.439	0.415	0.390	0.463	0.366	0.341	0.341	0.463	0.317	0																			
MANITOBA	0.341	0.293	0.366	0.317	0.439	0.317	0.195	0.293	0.366	0.268	0.293	0.341	0.463	0																		
MISSISSIPPI R.	0.341	0.293	0.366	0.415	0.439	0.268	0.341	0.341	0.317	0.268	0.439	0.390	0.317	0.341	0																	
MOTH L.	0.341	0.390	0.317	0.317	0.439	0.317	0.293	0.341	0.366	0.317	0.195	0.341	0.366	0.341	0.390	0																
RED R.	0.317	0.317	0.341	0.341	0.317	0.244	0.268	0.220	0.293	0.195	0.220	0.268	0.390	0.122	0.366	0.268	0															
ST. MARY'S R.	0.415	0.317	0.293	0.341	0.415	0.195	0.220	0.317	0.341	0.293	0.171	0.317	0.537	0.317	0.415	0.220	0.244	0														
SUPERIOR	0.390	0.341	0.317	0.415	0.439	0.220	0.293	0.341	0.317	0.268	0.244	0.341	0.463	0.341	0.341	0.293	0.268	0.073	0													
UPPER ROCK L.	0.439	0.537	0.415	0.463	0.341	0.463	0.585	0.537	0.415	0.561	0.488	0.341	0.366	0.683	0.431	0.537	0.610	0.512	0.488	0												
WESTMAN L.	0.463	0.366	0.341	0.293	0.317	0.244	0.317	0.317	0.341	0.293	0.220	0.366	0.439	0.268	0.365	0.268	0.244	0.293	0.317	0.561	0											
WHIRLPOOL L.	0.390	0.439	0.317	0.366	0.390	0.268	0.341	0.341	0.317	0.366	0.293	0.293	0.268	0.341	0.390	0.341	0.268	0.366	0.341	0.390	0.317	0										
MICHIGAN	0.366	0.366	0.244	0.341	0.366	0.390	0.366	0.366	0.341	0.390	0.366	0.268	0.390	0.366	0.317	0.415	0.293	0.390	0.415	0.463	0.439	0.415	0									
FRAINS L.	0.390	0.439	0.220	0.317	0.390	0.366	0.439	0.537	0.366	0.463	0.341	0.293	0.415	0.488	0.390	0.341	0.415	0.366	0.390	0.341	0.366	0.390	0.268	0								
BASELINE L.	0.341	0.293	0.768	0.268	0.439	0.268	0.195	0.439	0.268	0.317	0.293	0.390	0.512	0.244	0.293	0.244	0.268	0.220	0.244	0.537	0.366	0.439	0.317	0.341	0							
OHRID	0.512	0.415	0.488	0.488	0.512	0.488	0.463	0.512	0.537	0.488	0.415	0.463	0.537	0.463	0.463	0.366	0.488	0.390	0.415	0.561	0.439	0.512	0.537	0.415	0.512	0						
GOCZAKOWICE R.	0.390	0.390	0.366	0.463	0.488	0.463	0.439	0.488	0.415	0.463	0.488	0.293	0.366	0.341	0.439	0.390	0.366	0.415	0.439	0.439	0.463	0.341	0.366	0.293	0.390	0.366	0					
WINDERMERE	0.366	0.463	0.244	0.293	0.366	0.390	0.415	0.415	0.293	0.439	0.268	0.317	0.390	0.415	0.512	0.317	0.341	0.390	0.415	0.415	0.390	0.366	0.293	0.268	0.366	0.537	0.415	0				
RYDAL WATER	0.341	0.390	0.220	0.317	0.390	0.317	0.390	0.390	0.268	0.366	0.390	0.293	0.463	0.293	0.293	0.488	0.317	0.415	0.390	0.488	0.366	0.390	0.220	0.293	0.341	0.512	0.341	0.220	0			
ESTHWAITE W.	0.488	0.439	0.415	0.415	0.439	0.366	0.341	0.488	0.463	0.561	0.390	0.488	0.561	0.390	0.537	0.488	0.463	0.366	0.439	0.390	0.512	0.439	0.415	0.439	0.390	0.463	0.341	0.317	0.341	0		
ELTERWATER	0.317	0.415	0.341	0.390	0.366	0.488	0.415	0.610	0.341	0.634	0.512	0.415	0.439	0.512	0.512	0.415	0.488	0.488	0.512	0.366	0.585	0.561	0.341	0.268	0.317	0.537	0.317	0.244	0.366	0.268	0	
ROSTHERNE M.	0.439	0.488	0.415	0.463	0.439	0.463	0.585	0.439	0.463	0.366	0.390	0.390	0.415	0.488	0.488	0.390	0.415	0.463	0.439	0.390	0.415	0.439	0.366	0.341	0.488	0.463	0.439	0.220	0.293	0.390	0.415	0
MEAN	*0.380*	*0.389*	*0.330*	*0.345*	*0.388*	*0.320*	*0.344*	*0.398*	*0.330*	*0.374*	*0.323*	*0.417*	*0.415*	*0.356*	*0.382*	*0.350*	*0.322*	*0.339*	*0.353*	*0.470*	*0.364*	*0.369*	*0.301*	*0.367*	*0.342*	*0.476*	*0.400*	*0.368*	*0.358*	*0.457*	*0.426*	*0.425*

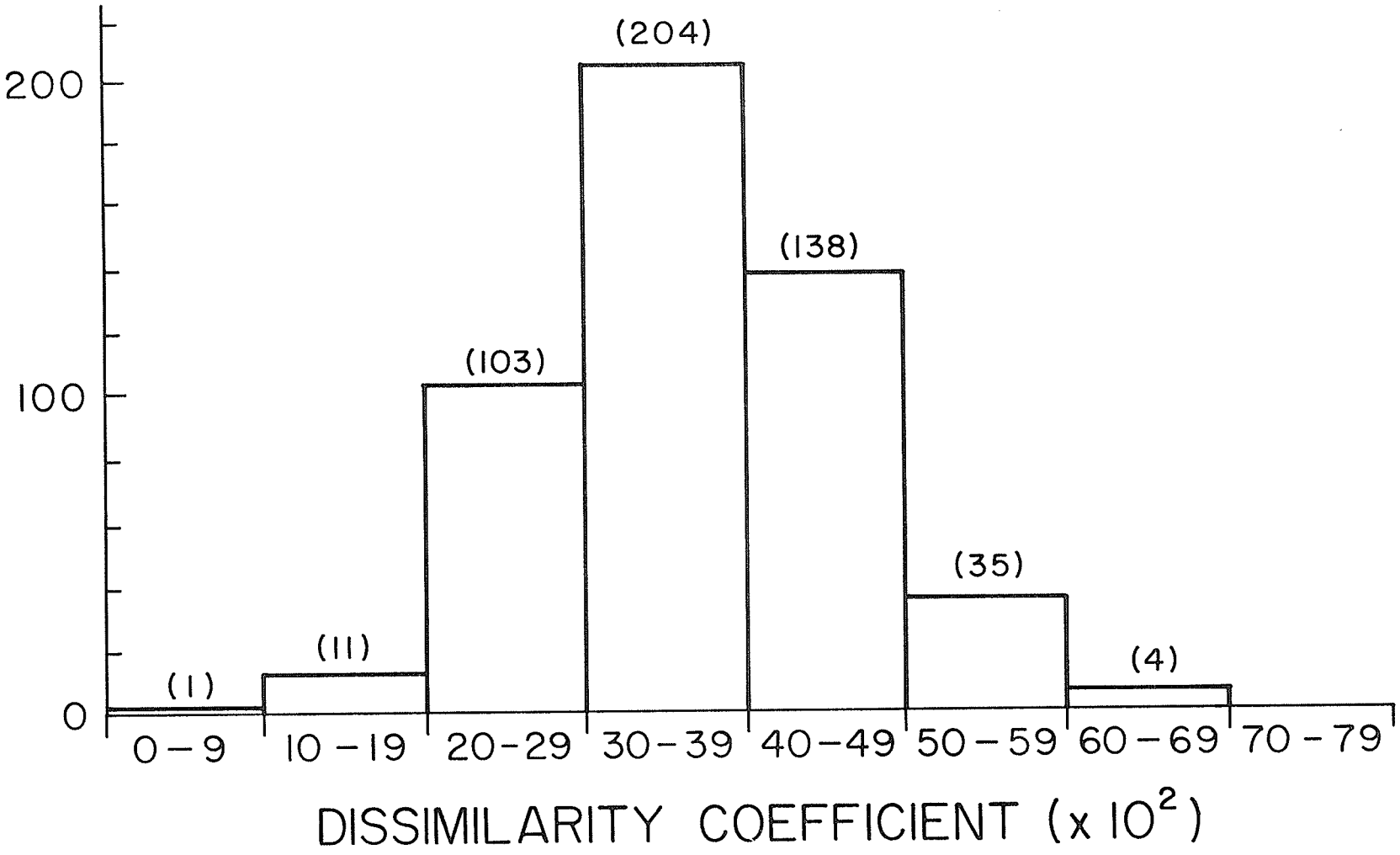


FIG. 2. FREQUENCY DISTRIBUTION OF DISSIMILARITY COEFFICIENT VALUES FOR 496 PAIRS OF POPULATION-SPECIFIC ELECTROPHORETIC BANDING PATTERNS.

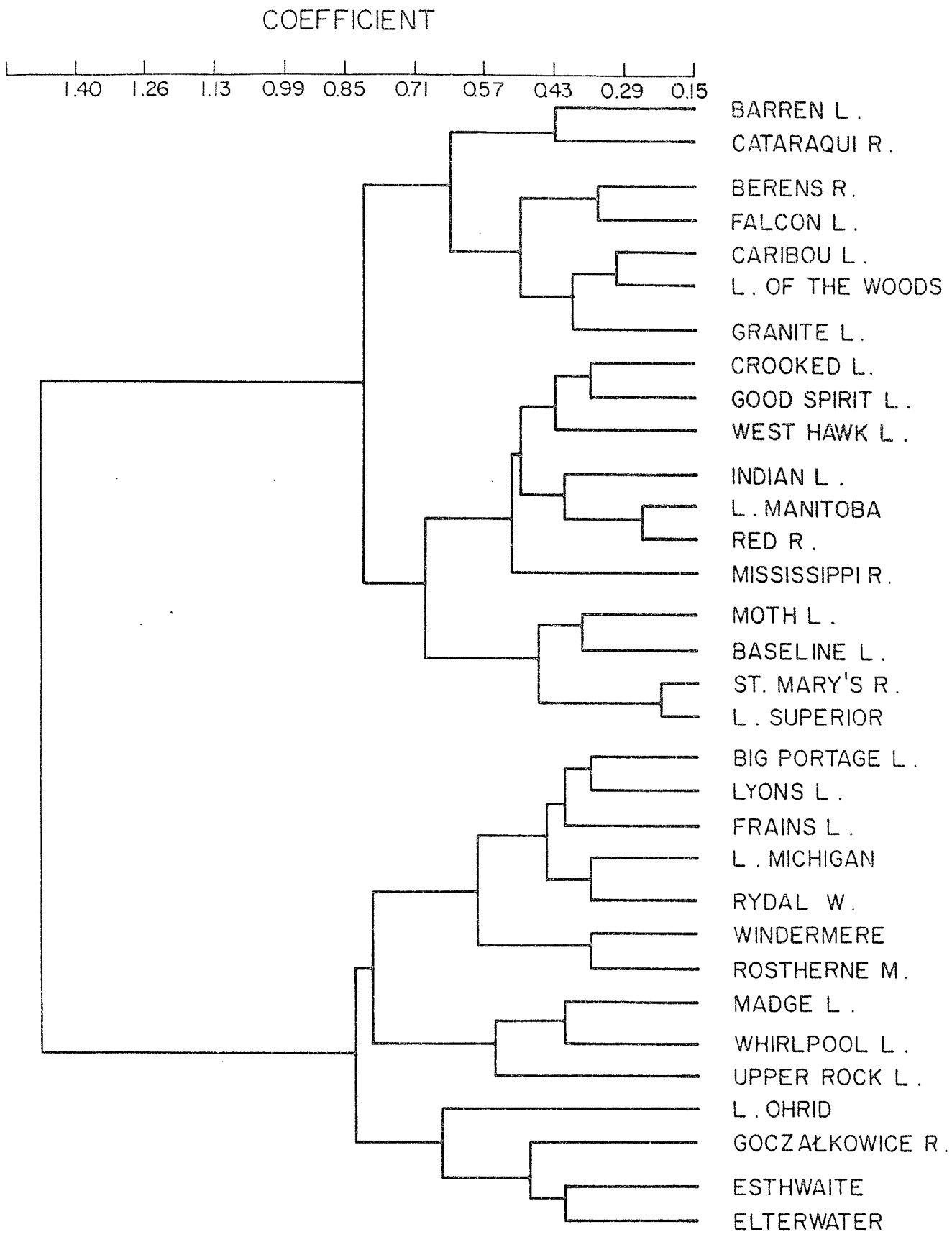


FIG. 3. DENROGRAM DEMONSTRATING HIERARCHICAL CLUSTERING OF POPULATION-SPECIFIC ELECTROPHORETIC BANDING PATTERNS.

Lake Ohrid and Upper Rock Lake.

An examination of the dendrogram at the two cluster stage reveals that the seven European populations are in one cluster which also contains a mixture of North American populations of diverse geographical and limnological origin. At the five cluster stage one cluster of seven consists of six Canadian Shield Lake populations from the Manitoba-Ontario border plus Cataraqui River in Eastern Ontario, another is comprised of four of the European populations, whereas the remaining three clusters include eclectic mixtures of populations.

DISCUSSION AND CONCLUSIONS

1. Population Structure

The lack of differences in electrophoretic banding patterns of both contemporaneous and non-contemporaneous multiple isolates of *A. formosa* indicates that populations of this species inhabiting individual lakes are clonal or at least highly homogeneous genetically.

This conclusion appears to be valid for small and/or morphometrically simple lakes. However, small genetic differences were found between isolates from Lake Superior at Rossport and those from St. Mary's River, which drains the lake some 150 km from the Rossport sampling site. It is thus highly probable that large lakes contain more than one phenotype restricted to different basins.

The observed lack of genetic heterogeneity in *A. formosa* populations is contrary to the assertion of Doyle (1975), who hypothesized that populations of planktonic algae consist of a multitude of competing genotypes. Likewise, it contradicts the findings of Lozeron (1902, in Körner, 1970), Allen (1921), Ruttner (1937), and Koob (1966), all of whom reported the presence of more than one strain in *A. formosa* populations.

However, these reports were based largely on the presence of discrete size classes in the populations. Such size classes are now known to form even in clonal cultures of *A. formosa* (Körner, 1970) through the production of dwarf cells by faulty cell division, a process entirely different from the progressive cell diminution according to the MacDonald-Pfitzer rule (von Stosch, 1965; Rao and Desikachary, 1970).

According to Canter and Jaworski (1978) the two Rydal Water clones used in the present study (L 277 and L 292) show different reactions to an isolate of the chytrid *Zygorhizidium affluens* but not an isolate of

Rhizophydium planktonicum. The difference in sensitivity to the former parasite indicates some genetic differences. However, it is likely to be caused by no more than a few genes and thus cannot be expected to be detected in a random sample of 41 protein bands.

2. Variation Among Populations

The experimental results clearly show that there are significant genetic differences among populations of A. formosa inhabiting different lakes.

It was not possible to interpret the observed isozyme and GP banding patterns genetically due to the lack of variation within populations and due to the high number of bands found. Moreover, there is no possibility of confirming or disproving genetic hypotheses by crossing experiments.

The large differences in the number of protein fractions detected in isolates from different populations are most likely caused by different degrees of heterozygosity (Grant and Proctor, 1980), which may or may not be fixed by polyploidy. Isolates heterozygous at loci that code for polypeptide subunits of randomly associating enzyme multimers would show the greatest number of bands (Scandalios, 1969).

It is concluded that the array of 32 population-specific electrophoretic banding patterns was not comprised of discrete groups. Had there been such groups, the frequency distribution of dissimilarity coefficient values of all possible pairs of patterns would have shown two modal classes, as it did in the study of 43 strains of the ciliate Tetrahymena pyriformis by Borden et al. (1973). In this case, the low dissimilarity coefficient mode was accounted for by pairs of strains belonging to the same phenoset, whereas the high dissimilarity coefficient mode was due to pairs of isolates belonging to different phenosets.

Correlation of the population-specific electrophoretic banding patterns with the geographical origins of the different populations was possible

only to a limited extent. The Lake Ohrid isolate was clearly genetically the most unique one, due to the great age and isolation of its habitat. At the five-cluster stage of cluster analysis it formed a grouping with Goczałkowice Reservoir, Esthwaite Water, and Elterwater populations, which are its nearest geographical neighbours but which are ecologically quite different.

Another grouping at the five-cluster stage included five lakes from the Manitoba-Ontario border (Barren L., Falcon L., Caribou L., Granite L., and L. of the Woods) in addition to the nearby Berens River and, surprisingly, the Cataraqui River from Southeastern Ontario.

In general, isolates from neighbouring lakes, even ones connected by a stream, showed no more similarity to each other than isolates from mutually distant lakes. For example, the three Southeastern Ontario populations (Cataraqui R., Indian L., and Upper Rock L.) are spread among three different clusters at the five-cluster stage. The English Lake District isolates are divided between two clusters at the five-cluster stage, as are the isolates from Southern Michigan.

Correlation of the electrophoretic banding patterns with the limnological characteristics of the habitats of the different populations is complicated by the fact that geographically close lakes usually have similar limnological characteristics.

There is no electrophoretic evidence for the existence of an oligotrophic ecotype because the four populations from oligotrophic environments (L. Ohrid, West Hawk L., L. Superior and St. Mary's R.) show no tendency to cluster together. However, it was observed during the course of the study that all isolates from the latter three sites were exceptionally difficult to isolate and grew very slowly compared to the rest of the culture collection. Lower maximum growth rates are typical of marine diatoms isolated from

oligrotrophic as opposed to eutrophic regions (Guillard and P. Kilham, 1977)

Likewise, there is no electrophoretic evidence for the existence of a eutrophic ecotype, since isolates from the nine populations from eutrophic habitats (Cataraqui R., Goczałkowice Res., Goodspirit L., Madge L., L. Manitoba, Mississippi R., Red R., Rostherne M., and Whirlpool L.) are spread among all clusters at the five-cluster stage. Only four of the above nine populations form pairs early in the clustering process. The similarity of the L. Manitoba to the Red R. isolates and the Madge L. to the Whirlpool L. isolates can in both cases be explained by geographical proximity, inclusion or exclusion in Glacial Lake Agassiz (Elson, 1967; Ritchie, 1967), or limnological similarity.

Isolates from mildly saline eutrophic prairie waters (Goodspirit L., Madge L., L. Manitoba, Red R., and Whirlpool L.) are split between two clusters at the five-cluster stage, again indicating that there is no distinct physiological race in these lakes.

In two of the eight lakes on the Manitoba-Ontario border (Barren L. and Lyons L.) A. formosa disappeared in early summer only to reappear in the fall. The Barren L. population is genetically similar to the rest of the populations which persisted in the warm epilimnion during the summer, while the Lyons Lake isolates show similarity to the Big Portage Lake population of Southern Michigan which also remained abundant in the summer. Consequently, it is not possible to explain the above similarities in electrophoretic banding patterns in terms of thermal races of the experimental species.

3. General Comments

The emerging picture of the population and species structure of A. formosa, i.e. little of any genetic variation within populations compared to genetic variation among different populations, is similar to that of the clubmoss (Lycopodium sp.), a living fossil that reproduces largely asexually (Levin and Crepet, 1973). Isolates of the common dandelion (Taraxacum officinale) from throughout the world also show a great deal of electrophoretically detectable genetic variation, despite the fact that this organism is an apomict (Solbrig, 1970). However, in this case populations consist of a mixture of genotypes (Solbrig and Simpson, 1977).

The habitat occupied by lacustrine phytoplankters such as A. formosa consists of semi-isolated "ecological islands", most of which have been available only since the end of the last Ice Age. Colonization and genetic differentiation in the intervening millenia have resulted in the species and population structure of today.

We do not know what maintains the genetic integrity of A. formosa populations despite a continuous inflow of water from other lakes containing foreign genotypes, in addition to other modes of transport. Nor do we know to what extent different populations of the experimental species are differentiated physiologically.

The results of the present study may have been influenced by the fact that only a small portion of the attempted isolations was successful, thus exposing the species to a selective pressure during the process of isolation. However, in view of the large number of replicate isolates from a large number of populations it is highly unlikely that this distortion would invalidate the resulting picture of the organism we call A. formosa.

REFERENCES

Allen,H.L. and B.T. Ocevski. 1976. Limnological studies in a large deep oligotrophic lake (Lake Ohrid,Yugoslavia). Arch. Hydrobiol.77: 1-21.

Allen,W.E. 1921. A quantitative and statistical study of the plankton of the San Joaquin River and its tributaries in and near Stockton, California. Univ. Cal. Publ. Zool. 22:1-292.

Antevs,E. 1929. Maps of the Pleistocene glaciations. Bull.Geol. Soc. Amer. 40: 631-720.

Atkinson, K.M. 1972. Birds as transporters of algae. Brit. Phycol.J. 7: 319-21

Bajkov,A.D. 1933. Report on fisheries investigations: Hydrobiological survey of West Hawk Lake. Man. Dept. Mines & Natur. Resources (Fisheries Branch) manuscript,36 pp.

Baker,A.F. and R.W. Holton. 1973. Electrophoretic analysis of proteins and malic dehydrogenase isozymes in nine oscillatorian bluegreen algae. Phycologia 12: 83-7.

Beam,C.A. and M. Himes. 1977. Sexual isolation and genetic diversification among some strains of Cryptothecodinium cohnii - like dinoflagellates. J. Protozool. 24: 532-9.

Belay,A. and G.E. Fogg. 1978. Photoinhibition of photosynthesis in Asterionella formosa (Bacillariophyceae). J. Phycol.14: 341-7.

Bombówna,M. 1962. Sedimentieren von Sinkstoffen in Staubecken-Goczałkowice. Acta Hydrobiol. 4: 69-118.

Borden,D., G.S. Whitt and D.L. Nanney. 1973. Electrophoretic characterization of classical Tetrahymena pyriformis strains. J. Protozool. 20: 693-700.

Borden,D., E.T. Miller, G.S. Whitt and D.L. Nanney. 1977. Electrophoretic analysis of evolutionary relationships in Tetrahymena. Evolution 31: 91-102.

Bringmann,G. and R. Kühn. 1971. Bestimmung der Begrenzungsfaktoren der Trophierung für die Kieselalga Asterionella formosa in West-Berlin Gewässern. Gesundh.-Ing. 92: 176-83.

Canter,H.M. and G.H.M. Jaworski. 1978. The isolation,maintenance,and host range studies of a chytrid(Rhizophidium planktonicum) parasitic on Asterionella formosa. Ann. Bot.(London) 42: 967-80.

Canter,H.M. and G.H.M. Jaworski. 1979. The occurence of a hypersensitive reaction in the planktonic diatom Asterionella formosa Hass. parasitized by the chytrid Rhizophidium planktonicum Canter in culture. New Phytol. 82: 187-206.

Canter,H.M. and J.W.G. Lund. 1948. Studies on plankton parasites. I. Fluctuations in the numbers of Asterionella formosa Hass. in relation to fungal epidemics. New Phytol. 47: 238-61.

Canter,H.M. and J.W.G. Lund. 1951. Studies on plankton parasites. III. Examples of interactions between parasitism and other factors determining the growth of diatoms. Ann. Bot.(London) 15: 359-72.

Carlson,R.E. 1977. A trophic state index for lakes. Limnol. Oceanogr. 22: 361-9.

Carpenter,E.J. and R.R.L. Guillard. 1971. Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology 52: 183-9.

Cheney,D.P. and G.R. Babbel. 1978. Biosystematic studies of the red algal genus Eucheuma. I. Electrophoretic variation among Florida populations. Mar. Biol.(Berlin) 47: 251-64.

Chu,S.P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. J. Ecology 30: 284-325.

Cober,J.-A. M.E. 1968. Interim report on Lake Manitoba water quality analysis 1966-1967. Man. Dept. Mines & Natur. Resources (Fisheries Branch), Manuscript report #68-2,23 pp.

Davis,B.J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404-27.

Derbyshire,E. and B.A. Whitton. 1968. A disc electrophoretic study of proteins of bluegreen algae. Phytochemistry 7: 1355-60.

Doyle,R.W. 1975. Upwelling,clone selection,and the characteristic shape of nutrient uptake curves. Limnol. Oceanogr. 20: 487-9.

Drebes,G. 1977. Sexuality. In:D. Werner. The biology of diatoms. Univ. of California Press. Berkeley.

Duthie,H.C.,M.L.Ostrofsky and M.R. Sreeniwasa. 1975. Freshwater algae from Western Labrador. I. Introduction & Bacillariophyta. Nova Hedwigia 26: 17-31.

Elson,J.A.1967. Geology of Glacial Lake Agassiz. In:W.J. Mayer-Oakes. Life,land,and water: Proceedings of the 1966 conference on environmental studies of the Glacial Lake Agassiz region. Univ. of Manitoba Press. Winnipeg.

Eppley,R.W. 1977. The growth and culture of diatoms. pp.24-64. In: D.Werner. The Biology of diatoms. Univ. of California Press. Berkeley.

Fisher,N.S. 1977. On the differential sensitivity of estuarine and open ocean diatoms to exotic chemical stress. Amer. Naturalist 111: 871-96.

Fisher,N.S.,L.B. Graham.E.J.Carpenter and Ch.F. Wurster. 1973. Geographic differences in phytoplankton sensitivity to PCBs. Nature 241: 548-9.

Foged,N. 1962. Notes on diatoms. III. Asterionella ralfsii. Bot. Tidsskr. 58: 68-71.

Gallagher,J.C.,1977. Population genetics of Skeletonema costatum (Grw.) Cleve over yearly cycles of abundance in Narragansett Bay. J. Phycol. 13: suppl. p. 23 (abstract).

Geitler,L. 1939. Auxospores in Synedra ulna. Planta 30: 551-66.

Gomori,G. 1955. Preparation of buffers for use in enzyme studies. pp.138-46. In: S.P. Colowick and N.O. Kaplan. Methods in enzymology,vol.I. Academic Press. New York.

Gooch,J.L. and T.J.M. Schopf. 1970. Population genetics of marine species of the phylum Ectoprocta. Biol. Bull. 138: 138-56.

Gottlieb,L.D. 1971. Gel electrophoresis: New approach to the study of evolution. BioScience 21: 939-44.

Grant,M.C. and V.W. Proctor. 1980. Electrophoretic analysis of genetic variation in the Charophyta. I.Gene duplication via polyploidy. J. Phycol. 16: 109-115.

Guillard,R.R.L. 1968. B_{12} specificity of marine centric diatoms. J. Phycol. 4: 59-64.

Guillard,R.R.L. and P. Kilham. 1977. The ecology of marine planktonic diatoms. pp.372-469. In:D. Werner. The biology of diatoms. Univ. of California Press. Berkeley.

Guillard,R.R.L., P. Kilham and T.A. Jackson, 1973. Kinetics of silica-limited growth in the marine diatom Thalassiosira pseudonana (Cyclotella nana). J. Phycol. 9: 233-7.

Guillard,R.R.L. and C.J. Lorenzen. 1972. Yellow-green algae with chlorophyllide c. J. Phycol. 8: 10-4.

Guillard,R.R.L. and J. H. Ryther. 1962. Studies of marine plankton diatoms. I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Grw. Can. J. Microbiol. 8: 229-39.

Hargraves,P.E. and R.R.L. Guillard. 1974. Structural and physiological observations on some small marine diatoms. Phycologia 13: 163-72.

Holton, R.W. 1973. Electrophoresis and the taxonomy of algae. Bull. Torrey Bot. Club 100: 297-303.

Huber-Pestalozzi, G. 1942. Das Phytoplankton des Süsswassers: Systematik und Biologie. Vol. 2, 2nd part (Diatomeen). E. Schweizerbart'sche Verlagsbuchhandlung. Stuttgart.

Hughes, J.C. and J.W.G. Lund. 1962. The rate of growth of Asterionella formosa Hass. in relation to its ecology. Arch. Mikrobiol. 42:117-29.

Hutchinson,G.E. 1967. A treatise on limnology. Vol. II: Introduction to lake biology and the limnoplankton. Wiley. New York.

Jaworski, G.H.M. and J.W.G. Lund. 1970. Drought resistance and dispersal of Asterionella formosa Hass. Beih. z. Nova Hedwigia 31: 37-48.

Jensen,A. and B. Rystad. 1974. Heavy metal tolerance of marine phytoplankton. I. The tolerance of three algal species to zinc in coastal waters. J. Exp. Mar. Biol. & Ecol. 15: 145-57.

Jørgensen,E.G. 1956. Growth inhibiting substances produced by algae. Physiologia Plant. 9: 712-26.

Kilham, S.S. 1975. Kinetics of silicon-limited growth in the freshwater diatom Asterionella formosa. J. Phycol.11: 396-9.

Knudsen,B.M. 1955. The distribution of Tabellaria in the English Lake District. Proc. Int. Assoc. Limnol. 12: 216-8/

Koob, D.D. 1966. Parasitism of Asterionella formosa Hass. by a chytrid in two lakes of the Rawah Wild Area of Colorado. J. Phycol. 2: 41-5.

Körner,H. 1970. Morphologie und Taxonomie der Diatomeengattung Asterionella. Nova Hedwigia 20: 557-724.

Kuehne,P.E. 1941. The phytoplankton of Southern and Central Saskatchewan. Canad. J. Research 19(C): 292-322.

Lehman,J.T.,D.B. Botkin and G.E. Likens. 1975. The assumptions and rationales of a computer model of phytoplankton population dynamics. Limnol. Oceanogr. 20: 343-64.

Levin,D.A. and W.L. Crepet. 1973. Genetic variation in Lycopodium lucidulum, a phylogenetic relict. Evolution 27: 622-32.

Lewin, J.C. and R.A. Lewin. 1960. Auxotrophy and heterotrophy in marine littoral diatoms. Can. J. Microbiol. 6: 127-34.

Lind, E.M. 1945. The phytoplankton of some Cheshire meres. Mem. and Proc. Manchester Lit. and Phil. Soc. 86: 83-105.

Lowry,O.H., N.J. Rosebrough,A. L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-75.

Lowe, C.W. 1924. The freshwater algae of Central Canada. Trans. Roy. Soc. Canada 18: 19-48.

Lund, J.W.G. 1949. Studies on Asterionella formosa Hass. I.The origin and nature of the cells producing seasonal maxima. J. Ecol.37:389-419.

Lund,J.W.G. 1950. Studies on Asterionella formosa Hass. II. Nutrient depletion and the spring maximum. J. Ecol. 38: 1-35.

Lund, J.W.G. 1957. Chemical analysis in ecology illustrated from Lake District tarns and lakes. II. Algal differences. Proc. Linn. Soc. London 167: 165-71.

Lund, J.W.G. 1959. Buoyancy in relation to the ecology of the freshwater phytoplankton. Brit. Phycol. Bull. 7: 1-17.

Lund, J.W.G. 1961. Asterionella formosa Hass. var acaroides Lemm.: A phycological enigma. Brit Phycol. Bull. 9: 63-6.

Lund, J.W.G. 1962. Phytoplankton from some lakes in Northern Saskatchewan and from Great Slave Lake. Can. J. Botany 40: 1499-1514.

Lund, J.W.G., G.H.M. Jaworski and H. Bucka. 1971. A technique for bioassay of freshwater with special reference to algal ecology. Acta Hydrobiol. 13: 235-49.

Lund, J.W.G., G.H.M. Jaworski and C. Butterwick. 1975. Algal bioassay of water from Blelham Tarn, English Lake District, and the growth of planktonic diatoms. Arch. Hydrobiol. 49 (supplement): 49-69.

Lund, J.W.G., F.J.H. Mackereth and C.H. Mortimer. 1963. Changes in depth and time of certain chemical and physical conditions and of the standing crop of Asterionella formosa Hass. in the North Basin of Windermere in 1947. Phil. Trans. Roy. Soc. 246(B): 255-90.

Macan, T.T. 1970. Biological studies of the English lakes. Longman Group Ltd. London.

Mackereth, F.J.H. 1953. Phosphorus utilization by Asterionella formosa Hass. J. Exptl. Botany. 4: 296-313.

Malinowski, K.C. 1974. Codium fragile- the ecology and population biology of a colonizing species. Ph. D. thesis. Yale University, New Haven.

Mallery, Ch.H. and N. Richardson. 1972. Disc gel electrophoresis of proteins of Rhodophyta. pp. 292-300. In: K. Nisizawa, Proceedings of the 7th International Seaweed Symposium. Univ. of Tokyo Press. New York.

Maurer, H.R. 1971. Disc electrophoresis and related techniques of polyacrylamide gel electrophoresis. Walter de Gruyter. Berlin.

McLeod, J.A. 1943. Preliminary biological investigation of eight lakes in the Whiteshell Forest Reserve. Man. Dept. Mines & Natur. Resources (Game and Fish. Branch). Manuscript. pp. 14.

Michalski, M.F.P., K.H. Nicholls and M.G. Johnson. 1975. Phosphorus removal and water quality improvements in Gravenhurst Bay, Ontario. Proc. Int. Assoc. Limnol. 19: 644-59.

Moed, J.R. 1973. Effect of combined action of light and silicon depletion on Asterionella formosa Hass. Proc. Int. Assoc. Limnol. 18: 1367-74.

Moss, B. 1972. Studies on Gull Lake, Michigan. II. Eutrophication- evidence and prognosis. Freshwater Botany 2: 309-20.

Murphy, L.S. 1978. Biochemical taxonomy of marine phytoplankton by electrophoresis of enzymes. II. Loss of heterozygosity in clonal cultures of the centric diatoms Skeletonema costatum and Thalassiosira pseudonana. J. Phycol. 14: 247-50.

Murphy, L.S. and R.A. Belastock. 1980. The effect of environmental origin on the response of marine diatoms to chemical stress. Limnol. Oceanogr. 25: 160-5.

Murphy, L.S., R.R.L. Guillard, H.-t. Lee and L.E. Brand. 1978. Distribution of electromorphs and growth rate characteristics in isolates of Thalassiosira pseudonana from the neritic - oceanic boundary. J. Phycol. 14: suppl. 26 (abstract).

Nelson, D.M., J.J. Goering, S.S. Kilham and R.R.L. Guillard. 1976. Kinetics of silicic acid uptake and rates of silica dissolution in the marine diatom Thalassiosira pseudonana. J. Phycol. 12: 246-52.

Nichols, H.W. 1973. Growth media - freshwater. pp. 7-24. In: J.R. Stein. Handbook of phycological methods: Culture methods and growth measurements. Cambridge Univ. Press. Cambridge.

Nipkow, F. 1927. Über das Verhalten der Skelette planktonischer Kieselalgen im geschichteten Tiefenschlamm des Zürich- und Baldeggersees. Z. Hydrol. Hydrogr. Hydrobiol 4: 71-120.

Nipkow, F. 1953. Die Auxosporenbildung bei Fragillaria crotonensis in Plankton des Zürichsees. Schweiz. Z. Hydrobiol 15: 302-10.

Oelshlegel, F. Jr. and M.A. Stahmann. 1973. The electrophoretic technique: A practical guide for its application. Bull. Torrey Bot. Club. 100: 260-71.

Ontario Ministry of the Environment. 1972. Water quality data for Ontario lakes and streams. Volume 7. pp.360.

Ontario Ministry of the Environment. 1974. Water quality data for Ontario lakes and streams. Volume 9. pp. 290.

Orloci,L. 1967. An agglomerative method for classification of plant communities. J. Ecology 55: 193-206.

Patrick, R. and Ch.W. Reimer. 1966. The diatoms of the United States exclusive of Alaska and Hawaii. Vol. I. Academy of Natur. Sciences. Philadelphia. pp. 688.

Pearsall, W.H.,C.H. Mortimer, J.W.G. Lund and E.D. LeCren. 1959. The work of the Freshwater Biological Association. Advanc. Sci.(London) 15: 521-38.

Peary, J.A. and R.W. Castenholz. 1964. Temperature strains of a thermophilic bluegreen alga. Nature (London) 202: 720-1.

Pennington, W. 1943. Lake sediments: The bottom deposits of the North Basin of Windermere with special reference to the diatom succession. New Phytologist 42: 1-27.

Rao, V.N.R. and T.V. Desikachary. 1970. The MacDonald - Pfitzer hypothesis and cell size in diatoms. Beih. z. Nova Hedwigia 31: 485-93.

Rawson, D.S. 1956. Algal indicators of trophic lake types. Limnol. Oceanogr. 1: 18-25.

Rawson, D.S. and J.E. Moore. 1964. The saline lakes of Saskatchewan. Can. J. Research 22(D): 141-201.

Ressler, N. 1973. A systematic procedure for the determination of the heterogeneity and nature of multiple electrophoretic bands. Anal. Biochem. 51: 589-610.

Richardson, N. and C.H. Mallery. 1973. Some chemosystematic aspects of the Audoudinella (Acrochaetium-Rhodochorton) complex. Amer. J. Botany 60: 1051-56.

Ritchie, J.C. 1967. Holocene vegetation of the Northwestern precincts of the Glacial Lake Agassiz basin. pp.217-29. In: W.J. Mayer-Oakes. Life, land, and water: Proceedings of the 1966 conference on environmental studies of the Glacial Lake Agassiz region. Univ. of Manitoba Press. Winnipeg.

Round, F.E. 1961. The diatoms of a core from Esthwaite Water. New Phytologist 60: 43-59.

Round, F.E. 1964. The diatom sequence in lake deposits: Some problems of interpretation. Proc. Int. Assoc. Limnol. 15: 1012-20.

Round, F.E. 1971. The growth and succession of algal populations in freshwaters. Mitt. Internat. Verein. Limnol. 19: 70-99.

Ruttner, F. 1937. Ökotypen mit verschiedener Vertikalvertailung im Plankton den Alpenseen. Int. Rev. Ges. Hydrobiol. Hydrogr. 35: 7-34.

Ryder, R.A. 1964. Chemical characteristics of Ontario lakes as related to glacial history. Trans. Amer. Fish. Soc. 93: 260-8.

Scandalios, J.G. 1969. Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genetics 3: 37-79.

Schelske, C.L. and J.C. Roth. 1973. Limnological survey of Lakes Michigan, Superior, Huron and Erie. Great Lakes Research Division. Publication # 17. Univ. of Michigan. Ann Arbor. pp.106.

Schoenberg, D.A. 1975. Gel electrophoresis patterns of Gymnodinium microadriaticum (Dinophyceae) from benthic marine invertebrates. J. Phycol. 11: suppl. 15 (abstract).

Schoenberg, D.A. 1977. Intraspecific variation in the algal partner of an algal-invertebrate symbiosis. J. Phycol.13: suppl. 61 (abstract).

Shaw, Ch.R. and R. Prasad. 1970. Starch gel electrophoresis: A compilation of recipes. Biochem. Genetics 4: 297-320.

Shechter, Y., J.W. Landau, N. Dabrowa and V.D. Newcomer. 1968. Biochemical taxonomy of the dermatophytes. I. Comparative disc electrophoresis of culture filtrate proteins. J. Investig. Dermatol. 51: 165-9.

Sheridan, R.P. 1976. Sun - shade ecotypes of a bluegreen alga in a hot spring. J. Phycol. 12: 279-85.

Sheridan, R.P. 1979. Seasonal variation in sun - shade ecotypes of Plectonema notatum (Cyanophyta). J. Phycol. 15: 223-6.

Smayda, T.J. 1974. Some experiments on sinking characteristics of fresh-water diatoms. Limnol. Oceanogr. 19: 628-35.

Solbrig, O.T. 1970. Genotype variation within and between populations of the common dandelion (Taraxacum officinale). Isozyme Bull. 3: 43-4.

Solbrig, O.T. and B.B. Simpson. 1977. Components of regulation of a popula-tion of dandelions in Michigan. J. Ecol. 62: 473-86.

Sonneborn, T.M. 1957. Breeding systems, reproductive methods and species problems in protozoa. pp.155-324. In: E. Mayr. The species problem. A.A.A.S. Symposium. Washington.

Sparling J.H. and C. Nalewajko. 1970. Chemical composition and phytoplankton of lakes in Southern Ontario. J.Fish.Res. Board Canada 27: 1405-28.

Sprenger, E. 1925. Asterionella gracillima Heib.in Grossteich bei Hirschberg in Böhmen. Arch. für Protistenkunde 52: 170-84.

Stankovic, S. 1960. The Balkan Lake Ohrid and its living world. Monogr. Biol. 9:1-357. W. Junk Publishers. The Hague.

Stockner, J.G. and W.W. Benson. 1967. The succession of diatom assemblages in the recent sediments of Lake Washington. Limnol. Oceanogr. 12: 513-32.

Stockner, J.G. and J.W.G. Lund. 1970. Live algae in postglacial lake deposits. Limnol. Oceanogr. 15: 41-58.

Stross, R.G. and S.M. Pemrick. 1974. Nutrient uptake kinetics in phytoplank-ton: A basis for niche separation. J. Phycol. 10: 164-9.

Swabey, Y.H. 1953. A plankton survey of three Rideau lakes. B. Sc. thesis. Queen's University. Kingston. pp.24.

Tait, A. 1970. Enzyme variation between syngens in Paramecium aurelia. Biochem. Genetics 4: 461-70.

Talling, J.F. 1955. The relative growth rates of threeplankton diatoms in relation to underwater radiation and temperature. Ann. Botany (London) 19: 329-41.

Talling, J.F. 1957 a . Photosynthetic characteristics of some freshwater planktonic diatoms in relation to underwater radiation. New Phytologist 56: 29-50.

Talling, J.F. 1957 b . The growth of two plankton diatoms in mixed cultures. Physiol. Plantarum 10: 215-23.

Thomas, D.L. and R.M. Brown Jr. 1970 a . New taxonomic criteria in the classification of Chlorococcum species. III. Isozyme analysis. J. Phycol. 6: 293-9.

Thomas, D.L. and R.M. Brown Jr. 1970 b . Isozyme analysis and morphological variation of thirty-two isolates of Protosiphon. Phycologia 9:285-92.

Thomas, D.L. and J.B. Delcarpio. 1971. Electrophoretic analysis of enzymes from three species of Chlamydomonas. Amer. J. Botany 58: 716-20.

Thomas, D.L. and R.D. Groover. 1973. Electrophoretic and immunological analyses of seven chlorosarcinacean algae. J. Phycol. 9: 289-96.

Thomas, J.F.J. 1959. Industrial water resources of Canada. Water Survey Report # 10 (Nelson River Drainage Basin in Canada, 1953-56).

Thomas, J.F.J. and R.M. Gale. 1965. Industrial water resources of Canada. Water Survey Report # 14 (The Upper Great Lakes Drainage Basin in Canada, 1957-1963).

Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. Ecology 58: 338-48.

Tilman, D. and S.S. Kilham. 1976. Phosphate and silicate growth and uptake kinetics of the diatoms Asterionella formosa and Cyclotella meneghiniana in batch and semicontinuous culture. J. Phycol. 12: 375-383.

Tilman, D., S.S. Kilham and P. Kilham. 1976. Morphometric changes in Asterionella formosa colonies under phosphate and silicate limitation. Limnol Oceanogr. 21: 883-6.

Titman, D. 1975. A fluorometric technique for measuring sinking rates of freshwater phytoplankton. Limnol. Oceanogr. 20: 869-75.

Titman, D. 1976. Ecological competition between algae: Experimental confirmation of resource-based competition theory. Science 192: 463-5.

Titman, D. and P. Kilham. 1976. Sinking in freshwater phytoplankton: Some ecological implications of cell nutrient status and physical mixing processes. Limnol. Oceanogr. 21: 409-17.

Tschermak-Woess, E. 1973. Über die bisher vergeblich gesuchte Auxosporenbildung von Diatoma. Österr. Bot. Z. 121: 23-7.

Underhill, P.A. 1977. Nitrate uptake kinetics and clonal variability in the neritic diatom Biddulphia aurita. J. Phycol. 13: 170-6.

Van Valkenburg, S.D. and R.E. Morris. 1970. The growth and morphology of the silicoflagellate Dictyocha fibula Ehrenberg in culture. J. Phycol. 6: 48-54.

von Stosch, H.A. 1965. Manipulierung der Zellgrösse von Diatomeen in Experiment. Phycologia 5: 21-4.

Ward, J.H.Jr. 1963. Hierarchical grouping to optimize an objective function. J. Amer. Stat. Ass. 58: 236-44.

Werner, D. 1977. Introduction with a note on taxonomy. pp.1-18. In: D. Werner. The biology of diatoms. Univ. of California Press. Berkeley.

Wesenberg-Lund, C. 1908. Plankton investigations of the Danish lakes.The Baltic freshwater phytoplankton, its origin and variation. Gyldendalske Boghandel. Copenhagen. pp.389.

Whipple, G.C. and D.D. Jackson, 1899. Asterionella: Its biology,its chemistry and its effect on water supplies. J. New England Waterworks Assoc. 14: 1-25.

Williams, L.G. and C. Scott. 1962. Principal diatoms of the major waterways of the United States. Limnol. Oceanogr. 7: 365-79.

Williams, W.D. 1969. Eutrophication of lakes. Proc. Royal Soc. of Victoria 83: 17-26.

Wishart, D. 1969. An algorithm for hierarchical classification. Biometrics 25: 165-70.

Wishart, D. 1978. CLUSTAN user manual. 3rd edition. Program Library Unit. Edinburgh Univ. Edinburgh.

Young, E.G. 1970. A comparison of the soluble proteins in various species of algae by disc electrophoresis in polyacrylamide gels. Phytochemistry 9: 2167-74.

Zhadin,V.I. and S.V. Gerd. 1963. Fauna and flora of the rivers,lakes and reservoirs of the U.S.S.R. U.S.S.R. Academy of Sciences. Moscow. (translated and distributed by Israeli Program for Scientific Translation).

APPENDIX I

Composition of Woods Hole MBL Growth Medium
(Guillard and Lorenzen 1972; Nichols 1973)

Stock Solution	NUTRIENT	Stock solution g/300 ml	Medium mg/l
#1	$CaCl_2.2H_2O$	11.03	36.76
#2	$MgSO_4.7H_2O$	11.09	36.97
#3	$NaHCO_3$	3.78	12.60
#4	K_2HPO_4	2.61	8.71
#5	$NaNO_3$	25.50	85.01
#6	$Na_2SiO_3.9H_2O$	8.53	28.42
#7	MICRONUTRIENTS		
	Na_2.EDTA	1.308	4.36
	$FeCl_3.6H_2O$	0.945	3.15
	$CuSO_4.5H_2O$	0.003	0.01
	$ZuSO_4.7H_2$	0.0066	0.022
	$CoCl_2.6H_2O$	0.003	0.01
	$MnCl_2.4H_2O$	0.054	0.18
	$Na_2MoO_4.2H_2O$	0.0018	0.006
	H_3BO_3	0.30	1.0
#8	VITAMINS		
	Thiamin - HCl	0.03	0.1
	Biotin (H)	0.00015	0.0005
	Cyanocobalamin (B_{12})	0.00015	0.0005
#9	TRIS	75	ca.0.5

All stock solutions are stored in dark bottles at 5-10 C except stock solution #8, which is stored frozen. The pH of stock solution #9 is adjusted to 7.2 with 1 N HCl. The growth medium is made up by adding 1 ml. of each of stock solution #1-8 and 2 ml of stock solution #9 to 990 ml of sterile distilled water.

APPENDIX II

Composition of stock solutions used for gel preparation and electrophoresis

(Davis, 1964; Maurer, 1971)

A. Separation gel (7.5%)

Stock solution I:	30.0 g acrylamide 0.8 g BiS H_2O to 100 ml
Stock solution II:	24 ml 1 N HCl 18.15 g TRIS 0.2 ml TEMED H_2O to 100 ml (pH = 8.9)
Stock solution III:	0.14 g ammonium persulfate H_2O to 100 ml
	Mixing ratio: I:II:III = 3.5 ml:3.5 ml: 7 ml

B. Stacking gel (3.1%)

Stock solution IV:	5.0 g acrylamide 1.25 g BiS H_2O to 100 ml
Stock solution V:	24 ml 1 N HCl 2.99 g TRIS 0.2 ml TEMED H_2O to 100 ml (pH = 6.7)
Stock solution VI:	2.0 mg riboflavin H_2O to 100 ml
	Mixing ration: IV:V:VI: = 2 ml: 1 ml: 1 ml

C. Homogenation Buffer: (same as solution V minus TEMED, diluted 4x)

24 ml 1 N HCl
2.99 g TRIS
H_2O to 400 ml (pH = 6.7)

D. Electrode (= Reservoir) Buffer:

6.0 g TRIS
28.8 g glycine
H_2O to 1 l (pH = 8.3)
dilute 10x prior to use.

APPENDIX III

Composition of enzyme assay mixtures

(Volumes are sufficient for staining 3 gels.
Buffers are those of Gomori (1955))

1. Acid phosphatase (after Scandalios, 1969)

0.060 g α-naphthyl acid phosphate
0.060 g Fast Blue R.R. Salt
0.060 g Fast Garnet GBC diazonium salt
12 drops 5% $MgCl_2$ + 5% $MnCl_2$ solution
Acetate buffer (pH = 4.0) to 60 ml

2. Alkaline phosphatase (after Scandalios, 1969)

0.060 g α-naphthyl acid phosphate
0.060 g Fast Blue R.R. Salt
1.2 g NaCl
12 drops 5% $MgCl_2$ + 5% $MnCl_2$ solution
TRIS buffer (pH=8.0) to 60 ml

3. Esterase (after Shaw & Prasad, 1970)

Substrate solution:
5 ml. H_2O
5 ml. acetone
0.10 g α-naphthyl acetate
0.10 g β-naphthyl acetate

1.8 ml. substrate solution
0.060 g Fast Blue R.R. Salt
Phosphate buffer (pH=6.0) to 60 ml.

4. Glutamate dehydrogenase (after Shaw & Prasad, 1970)

substrate solution: 16.9 g Na·glutamate
phosphate buffer (pH=7.0) to 100

9 ml. substrate solution
0.018 Nitro Blue Tetrazolium
0.030 g NAD
0.0012 PMS
TRIS buffer (pH = 9.0) to 60 ml

5. Malate dehydrogenase (after Shaw & Prasad, 1970)

substrate solution: 1.34 g L - malic acid
4.9 ml 2 M $Na_2CO_3 \cdot H_2O$
H_2O to 100 ml

6 ml substrate solution
0.018 g Nitro Blue Tetrazolium
0.030 g NAD
0.0012g PMS
TRIS buffer (pH = 9.0) to 60 ml.

6. Leucine aminopeptidase (after Mallery & Richardson, 1972)

substrate solution: 0.05g L-leucyl-β-naphthylamide
10 ml acetone
40 ml water

6 ml substrate solution
phosphate buffer (pH = 6.0) to 60 ml
Incubate for 1 hour, then rinse in
distilled H_2O and incubate again in:

0.030 g Fast Garnet GBC diazonium salt
phosphate buffer (pH = 6.0) to 60 ml.