Résumé. On souligne l'importance d'un procédé convenable pour analyser la variation géographique. L'efficacité d'une méthode multivariante de la taxonomie numérique (l'analyse canonique) en réduisant une situation compliquée et hypermultivariable dans un modèle oligovariant intelligible est indiqué. On montre comment *N. natrix* donne l'exemple de la naissance d'espèces (influence de la calotte glaciaire) et de l'apport variable de gènes communs et on propose aussi de restreindre l'emploi de sous-espèces pour une espèce naissante.

R. S. THORPE

Department of Biological Sciences, City of London Polytechnic, 31 Jewry Street, London EC3N 2EY (England), 25 June 1974.

Influence of pH and Concentration of Phosphate Ions on Growth and Nitrogen Fixation in a Blue-Green Alga *Cylindrospermum majus*

As very little information is available on the factors controlling growth and nitrogen fixation by the heterocystous blue-green algae under culture conditions, it was considered desirable to study the effects of varying pH values and phosphate-ion concentrations and to examine statistically their significance, if any, on growth and nitrogen fixation by *Cylindrospermum majus*.

Cylindrospermum majus Kützing, a filamentous and nitrogen-fixing blue-green alga belonging to the family Nostocaceae, was collected from paddy fields of Bhupalsagar (Udaipur). It was isolated first in unialgal culture and then made bacteria-free by UV-irradiation. It was grown for a period of 30 days in conical flasks containing 100 ml medium of ALLEN and ARNON[1]. The pH of the culture medium was adjusted to different levels of 6.0, 8.0 and 10.0 after autoclaving and subsequent cooling of medium. In another experiment, the alga was grown in increasing concentrations of phosphate ions (50, 100, 250 and 500 mg/l) in the medium. The pH of the medium was maintained at 7.5 in all cases in which effects of

Dry weights and nitrogen contents of *C. majus* at different pH values and phosphate ion concentrations along with statistical analysis of data

pH of culture medium	Phosphate (mg/1)	Dry wt. of alga (mg)	N content of alga		N content of culture filtrate (mg)	Total N	
			(mg)	(%)		(mg)	(%)
6.0		21.1	0.917	4.40	0.378	1.295	6.21
8.0		14.5	0.805	5.69	0.246	1.051	7.45
10.0		45.5	1.169	2.56	0.441	1.610	3.53
	Without PO_4^{3-}	7.1	0.399	6.08	0.091	0.490	7.46
	50.0	9.5	0.868	9.10	0.175	1.043	10.96
	100.0	12.1	1.198	9.98	0.322	1.519	13.02
	250.0	27.4	1.771	6.46	0.686	2.458	8.96
	500.0	24.8	1.113	4.55	0.077	1.190	4.91
S.E. Mean ±		3.971	0.2560	0.9319	0.0466	0.3026	1.2018
C.D. 5%		13.28	N.S.	N.S.	N.S.	N.S.	N.S.
C.D. 1%		19.65	N.S.	N.S.	N.S.	N.S.	N.S.
	C.D. 5%	13.28	N.S.	3.117	0.156	1.012	4.020
	C.D. 1%	N.S.	N.S.	N.S.	0.231	N.S.	N.S.

Analysis of variance

Source of variation	d.f.	Dry wt. of alga (mg)		N content of alga				N content of culture filtrate		Total N			
				(mg)		(%)		(mg)		(mg)		(%)	
		M.S.S.	V.R.	M.S.S.	V.R.	M.S.S.	V.R.	M.S.S.	V.R.	M.S.S.	V.R.	M.S.S.	V.R.
Replications	1	17.01	0.539	0.104023	0.794	1.0506	0.605	0.004356	1.000	0.012996	0.017	1.5314	0.530
pH	2	533.30	16.900[a]	0.069514	0.530	4.9341	2.841	0.019806	4.547	0.157080	0.858	8.0116	2.773
PO_4^{3-}	4	172.52	5.470[b]	0.500004	3.810	10.0785	5.803[b]	0.127038	29.164[a]	1.057587	5.776[b]	19.5430	6.764[b]
pH PO_4^{3-} interaction	1	440.92	13.980[a]	0.042321	0.323	34.0658	19.613[a]	0.026966	6.191[b]	0.001707	0.009	41.6250	14.407[a]
Error	7	31.54		0.131098		1.7369		0.004356		0.183097		2.8892	
Total	15												

The given values are averages of 2 replicates.
V.R., variance ratio; [a] significant at 1% level; [b] significant at 5% level.

different concentrations of phosphate ions were studied. The solution of K_2HPO_4 was autoclaved separately to avoid precipitation in the basal medium. All the culture flasks were incubated under fluorescent light with an intensity of 1.5×10^5 erg/cm²/sec at a temperature of 30 ± 2°C. Growth of the alga was estimated on dry weight basis. Nitrogen contents of the alga as well as of culture filtrates were determined by semi-micro-Kjeldahl method. A selenate catalyst mixture was used during digestion and ammonia distilled in a Markham still.

The Table shows dry weights and nitrogen contents of *C. majus* at different pH values and phosphate-ion concentrations. A perusal of the data shows that the alga favoured a relatively high pH for its maximum growth, whereas percentage of total nitrogen fixed was highest at pH 8.0. Also, growth and nitrogen fixation were improved by increasing concentration of phosphate ions upto a certain level. Maximum nitrogen fixation on per cent basis was recorded up to phosphate concentration of 100 mg/l only.

The data obtained were subjected to statistical analysis. It was found that pH of the culture medium affected the algal growth significantly but no significant effect of different pH values on nitrogen fixation was observed. However, different concentrations of phosphate ions and their interactions with pH of the basal medium were found to be quite significant for growth and total nitrogen fixed by the alga.

Of the different kinds of algae found in paddy fields of India, the blue-greens are most abundant. Many of them are nitrogen fixers and play an important role in the nitrogen economy of soils. It is generally recognized that the nitrogen-fixing blue-green algae liberate appreciable quantities of fixed nitrogen into the medium during healthy growth. Most of this extracellular nitrogen is in the form of peptides, whereas free amino acids are present only in small amounts (FOGG[2]). Therefore, the real importance of nitrogen fixation by the blue-green algae in nature lies in the fact that fixed nitrogen becomes available for growth of the associated non-nitrogen-fixing plants. In fact, STEWART[3] has found evidence of a direct transfer of previously fixed nitrogen by a species of *Nostoc* to the associated non-nitrogen-fixing plants.

Nitrogen fixation by blue-gree algae is maximum only in the slightly alkaline range and any deviation on either side of this range depresses nitrogen fixation significantly. The fixation falls off markedly below and above the pH range of 7.0–8.0. FAY and FOGG[4] studied the effects of hydrogen-ion concentration on *Chlorogloea fritschii* and found no differential effects of pH on growth and nitrogen fixation. COBB and MYERS[5] also did not find any significant effect of pH on nitrogen fixation by *Anabaena cylindrica*. STEWART[6] has suggested that the effect of pH is probably on metabolism in general and not specifically on nitrogen fixation.

While considering the uptake of phosphate ions, pH of the medium is very important as it may alter the rate of phosphate uptake either by a direct effect on permeability of the cell membrane or by changing ionic form of the phosphate (EPSTEIN[7]). Our results indicate that at relatively low concentrations of exogenously supplied phosphate ions or even in a medium entirely devoid of them, growth and amounts of nitrogen fixed by *C. majus* were quite considerable. But higher concentrations of phosphate ions were not found conducive to the process of nitrogen fixation, the exact reason of which is not clearly understood.

Zusammenfassung. An der auf den indischen Reisfeldern heimischen blaugrünen Alge *Cylindrospermum majus* wurde der Einfluss von Änderungen der pH- und PO^3_4-Konzentration auf Wachstum und Stickstoff-Fixation untersucht. Während pH-Änderungen sich nur auf das Wachstum auswirkten, hatte Erhöhung der PO^3_4-Konzentration bis zu einem gewissen Bereich (bei konstantem pH) sowohl signifikante Wachstumssteigerung als auch Stickstoffeinbau zur Folge. Die Bedeutung der Stickstoff-fixierenden niederen Algen als Stickstoffquelle für andere Pflanzen wird diskutiert.

V. K. SHARMA and H. D. KUMAR[8]

Department of Botany, School of Basic Sciences and Humanities, University of Udaipur, Udaipur 313001 (India); and Department of Botany, Banaras Hindu University, Varanasi 221005 (India),
24 June 1974.

[1] M. B. ALLEN and D. I. ARNON, Pl. Physiol. *30*, 366 (1955).
[2] G. E. FOGG, Proc. R. Soc. B. *139*, 372 (1952).
[3] W. D. P. STEWART, Nature, Lond. *214*, 603 (1967).
[4] P. FAY and G. E. FOGG, Arch. Mikrobiol. *42*, 310 (1962).
[5] H. D. COBB JR. and J. MYERS, Am. J. Bot. *51*, 733 (1964).
[6] W. D. P. STEWART, Proc. R. Soc. B. *172*, 367 (1969).
[7] E. EPSTEIN, in *Handbuch der Pflanzenphysiologie* (Ed. W. RUHLAND; Springer Verlag, Berlin 1956), vol. 2, p. 398.
[8] Department of Botany, Banaras Hindu University, Varanasi 221005, India.

The Glair Glands and Oosetae of *Austropotamobius pallipes* (Lereboullet)

The origin and function of the glair exuded by the crayfish just prior to egg laying is obscure. In this connection a number of studies have been made of spawning in the European[1], Australian and American crayfishes, but not of *A. pallipes*, the endemic British species.

In mid-September, sexually mature females of *A. pallipes* are conspicuous by the presence of creamy-white patches on the pleura, and sterna of the abdomen, and on the pleopods and uropods, but never on the telson. A closer examination of these cream coloured areas, using the scanning electronmicroscope, discloses the presence of numerous pores in the overlying integument (Figure 1a). These pores are grouped together in roughly circular patches, presenting the appearance of 'pepper-pot' tops (Figures 1b, c and d). Observations in the field show that it is through these pores that the glair is exuded (Figure 1e), and the cream colouration is due to the very large groups of glair glands underneath the areas of perforated integument. On the pleopod the pores occur in much smaller groups (Figure 1f).

In *A. pallipes* the distribution pattern of the pores is constant; they are very numerous on the anterior faces of the pleura, less so on the anterior faces of the protopodites, and first segments of the endopodites and

[1] Z. MALACZYNSKA-SUCHCITZ, Bull. Soc. scient. Lett., Poznan, *13B*, 39 (1956).