

parisons of glucose metabolism, that cultures are carefully matched for age in vivo and in vitro, preferably derived from skin (the best tissue for routine biopsy) from a single anatomical site and from postnatal donors of known predisposition to diabetes.

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**Résumé.** Les fibroblastes humains utilisent une quantité de glucose plus grande pendant leur croissance logarithmique que dans leur phase stationnaire. Les cultures à passage tardif consomment plus de glucose par cellule que les cultures à passage hâtif sans égard à la phase. Ce système doit être utile pour rechercher les altérations métaboliques au cours du vieillissement cellulaire et pour découvrir la base biochimique de certaines maladies dépendant de l'âge.

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### Biometric Analysis of Incipient Speciation in the Ringed Snake, *Natrix natrix* (L.)

*N. natrix* shows a considerable amount of infraspecific variation and a large number of subspecies have been recognized<sup>1-4</sup> based on only a few characters, an inadequate number of specimens and without proper consideration of the non-geographic infraspecific variation.

This paper is an attempt to make a more objective and comprehensive assessment of the status of the various populations by using univariate and multivariate biometric methods to analyze the complicated patterns of geographic variation and univariate methods to detect and negate the effect of the non-geographic infraspecific variation such as allometric growth and sexual dimorphism.

750 specimens representing the entire range of the species were studied and the range of the species was then divided into approximately 50 small relatively homogeneous geographic units (on the basis of collecting gaps, physical isolation, and the overall similarity of specimens as assessed by cluster analyses) so that the geographic variation between the geographic units and the non-geographic infraspecific variation within the units could be analyzed.

Of the 160 characters<sup>5</sup> originally recorded from the scalation, colour pattern, internal anatomy, dentition, dermal sense organs and body proportions, 52 (50%) characters were chosen for the canonical analyses; the rest being discarded because they did not show significant geographic variation (as assessed by a one-way analysis of variance), did not contribute original information (i.e. had a high pooled within-group correlation to other included characters) or for other taxonomic or statistical reasons.

Canonical analysis simultaneously takes into account the variation and covariation of all of the characters and computes discriminant axes so as to produce the minimum overlap between the geographic units. This method, together with the related D<sup>2</sup> analyses, clearly indicates that the geographic units largely cluster into 3 groups, i.e., an eastern cluster, a western cluster and a Tyrrhenian cluster (Figures 1 and 2). However, the specimens from the Bologna area of north Italy are phenetically and geographically intermediate between the eastern and western specimens and the Coriscan specimens are phenetically intermediate between the Sardinian and western mainland specimens.

It should be noted that a detailed investigation of all the available canonical axes, together with further multivariate analyses of the variation within the eastern and western clusters indicate that these 2 groups are not homogeneous but show a considerable amount of complex geographic variation.

The question arises as to how the 2 mainland populations could have diverged from one another because the eastern and western population are adjacent to one another in Central Europe and are not separated by any physiographic barrier. The contact zone between these 2 populations does not completely coincide with the River Rhine or mountains to the east as previously implied<sup>1,2</sup> and, moreover, since *N. natrix* is both fairly aquatic and found in mountainous regions the Rhine and associated mountains cannot be considered as effective barriers to gene flow.

It is suggested that during the extension of the Pleistocene ice caps the population of *N. natrix* would have been split so that separate south western and south eastern

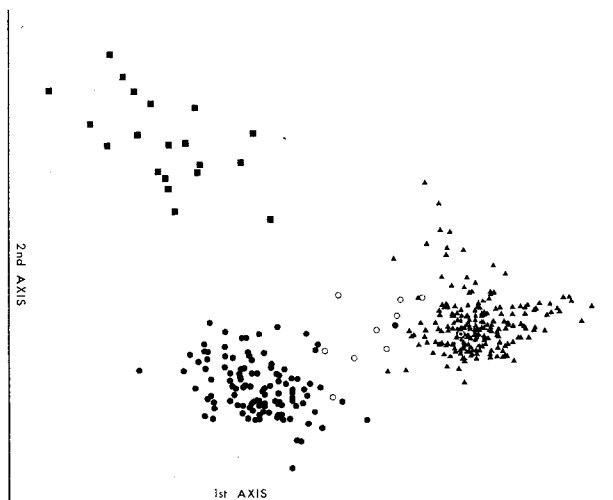


Fig. 1. Canonical analysis. Female specimens, 48 characters. First axis has 33.3% discrimination. Second axis has 16.5% discrimination. Solid circles, western specimens; triangles, eastern specimens; squares, Tyrrhenian (Corsica and Sardinia) specimens; open circles, Bologna area specimens.

<sup>1</sup> G. HECHT, Mitt. Zool. Mus. Berlin 16, 244 (1930).

<sup>2</sup> R. MERTENS, Abh. senckenb. naturforsch. Ges. 476, 1 (1947).

<sup>3</sup> R. MERTENS, Abh. senckenb. naturforsch. Ges. 38, 175 (1957).

<sup>4</sup> R. MERTENS, Abh. senckenb. naturforsch. Ges. 47, 117 (1966).

<sup>5</sup> R. THORPE, J. Linn. Soc. (Biol.), in press.

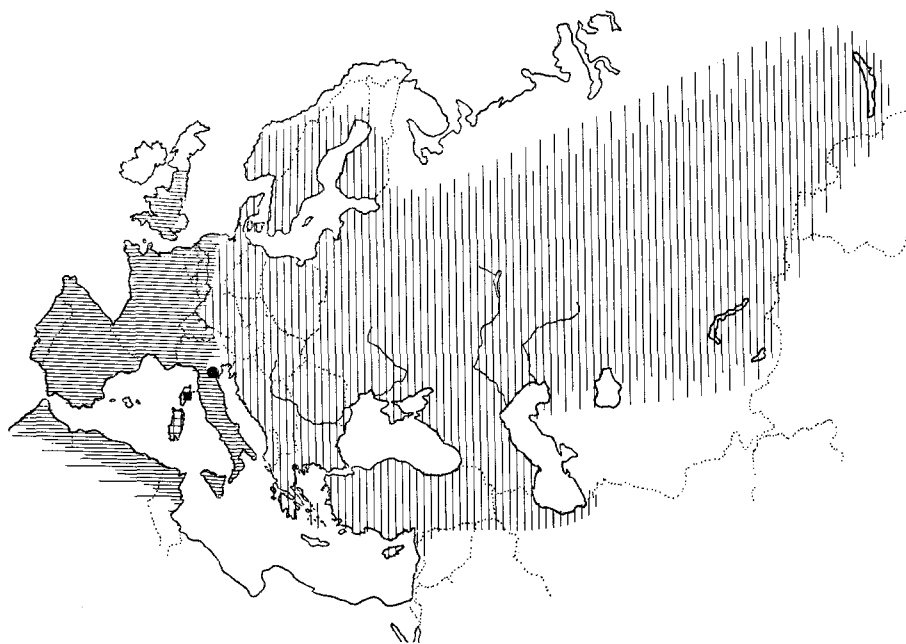


Fig. 2. Approximate range of the 3 subspecies of *N. natrix*. Vertical lines, *N. natrix natrix*; horizontal lines, *N. natrix helvetica*; squares, *N. natrix cetti*; solid circle, area of introgression, *N. n. natrix* × *N. n. helvetica*; solid squares, Corsican intermediates, *N. n. helvetica* — *N. n. cetti*.

refuge populations resulted. The two hypothesized refuge populations would have to have been separated for long enough and completely enough to diverge and establish distinct co-adapted gene pools to explain why these 2 populations have largely retained their integrity on coming together again in post-glacial times.

Examples of other vertebrates showing this east-west pattern of differentiation are known such as crows<sup>6</sup> and hedgehogs<sup>7</sup> and also the closely related snake species *Natrix tessellata* and *N. maura*. *N. maura* (western) and *N. tessellata* (eastern) appear to have speciated when the Pleistocene ice caps split the ancestral species into eastern and western components. Since *N. maura* and *N. tessellata* are more southerly in their distribution the refuge populations would probably have been separated for longer than those of *N. natrix* and this is possibly why they have achieved a greater degree of reproductive isolation.

However, whilst the present state of knowledge provided by this study indicates that the eastern and western populations of *N. natrix* may not have completely speciated, the specimens adjacent to the zone of contact (except at Bologna) remain distinct even though they are geographically close. This indicates a limited amount of gene flow and suggests incipient speciation. The intermediate specimens from Bologna (Figure 1) indicate introgressive hybridization in this limited area. Therefore *N. natrix* not only provides an example of incipient speciation but also an example of different degrees of introgression along a contact zone.

The taxonomic status of the western and eastern population is open to question. In Switzerland and S.W. Germany the western and eastern forms make a close approach but only a few intermediates have been identified whereas in north western Germany the relationship between the 2 forms is uncertain because the localities samples are too widely spaced.

Because of the occurrence of intermediates (in a multivariate sense) and univariate evidence of gene flow between the eastern and western populations, I provi-

sionally regard them as subspecies, with *N. natrix natrix* (Linnaeus, 1758) the correct trinomial for the eastern population and *N. natrix helvetica* (Lacépède, 1789) the correct trinomial for the western population. The Bologna specimens may be regarded as 'intersubspecific' hybrids. Subspecies in this context are grossly different populations defined by the criteria of incipient speciation.

Current work does not indicate that the eastern and western populations would more suitably be regarded as species but the matter should not be regarded as closed.

*N. natrix* provides an excellent example of the inability of formal trinomial nomenclature to adequately deal with divergent isolated populations. The Sardinian populations are too distinct to be included in with the mainland subspecies and are therefore allocated a trinomial of their own, *N. natrix cetti* (Gene, 1838). Since the Corsican population is phenetically intermediate between the western and Sardinian populations it is referred to here as *N. natrix helvetica*-*N. natrix cetti* and whilst this may be unconventional it is the most logically consistent option.

The problems of trinomial nomenclature and an informal alternative to the above mentioned 3 subspecies are discussed in a full account of this research which is to appear elsewhere<sup>8</sup>.

<sup>6</sup> W. MEISE, J. Orn., Lpz. 76, 1 (1928).

<sup>7</sup> G. CORBET, Symp. zool. Soc., Lond. 26, 105 (1970).

<sup>8</sup> I wish to thank the staff of the many European Museums who loaned me specimens; Dr. E. KRAMER who loaned me his personal collection; Dr. E. ARNOLD, Miss GRANDISON and Dr. M. HILLS of the British Museum (Natural History) for their assistance; and particularly, Dr. G. UNDERWOOD for his aid, encouragement and influence. Dr. G. UNDERWOOD obtained S.R.C. support for the latter part of this research and the computing facilities were provided by the University of London Computer Centre.

**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.

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## 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* Kützinger, a filamentous and nitrogen-fixing blue-green alga belonging to the family Nostocaceae, was collected from paddy fields of Bhupal-

sagar (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<sup>1</sup>. 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/l)	Dry wt. of alga (mg)	N content of alga		N content of culture filtrate (mg)	Total N							
			(mg)	(%)		(mg)	(%)						
6.0	Without PO <sub>4</sub> <sup>3-</sup> 50.0 100.0 250.0 500.0	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						
		7.1	0.399	6.08	0.091	0.490	7.46						
		9.5	0.868	9.10	0.175	1.043	10.96						
		12.1	1.198	9.98	0.322	1.519	13.02						
		27.4	1.771	6.46	0.686	2.458	8.96						
		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 <sup>a</sup>	0.069514	0.530	4.9341	2.841	0.019806	4.547	0.157080	0.858	8.0116	2.773
PO <sub>4</sub> <sup>3-</sup>	4	172.52	5.470 <sup>b</sup>	0.500004	3.810	10.0785	5.803 <sup>b</sup>	0.127038	29.164 <sup>a</sup>	1.057587	5.776 <sup>b</sup>	19.5430	6.764 <sup>b</sup>
pH PO <sub>4</sub> <sup>3-</sup> interaction	1	440.92	13.980 <sup>a</sup>	0.042321	0.323	34.0658	19.613 <sup>a</sup>	0.026966	6.191 <sup>b</sup>	0.001707	0.009	41.6250	14.407 <sup>a</sup>
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; <sup>a</sup> significant at 1% level; <sup>b</sup> significant at 5% level.