Skeletal polymorphism and genetic drift in a Delhi frog, Rana cyanophlictis*†

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1. INTRODUCTION

Quasi-continuous minor skeletal variations have been described and used to study the inter and intra strain differences in the laboratory mouse *Mus musculus* (Grüneberg, 1950, 1954; Grewal, 1962*a*; Deol, 1955; Deol *et al.*, 1957). This work revealed that the presence or absence of a particular variant in an individual depends to a great extent on its genetic make-up. The genetic basis is multifactorial and may sometimes be partly connected with the state of homozygosity of the stock.

The study of epigenetic polymorphism extended to wild populations of mice as well as other rodents (Weber, 1950; Deol, 1958; Harland, 1958; Grüneberg, 1961; Berry & Searle, 1963; Berry, 1963; Grüneberg *et al.*, 1966) has revealed considerable inter-population differences.

The systematic studies of such threshold variants have so far been confined to rodents in general and mice and rats in particular. Information regarding skeletal variants in any other animal group is scarce. Nobel (1954) noted the incidence of vertebral fusion, sacralization of thoracic vertebrae and variation in the number of trunk vertebrae in anurans.

The present paper describes nineteen such discontinuous variants found in the skeleton of the skipper frog, *Rana cyanophlictis*, and these variants have been used as a tool to analyse diversity among populations of Delhi frogs.

2. MATERIAL AND GENERAL METHODS

Rana cyanophlictis is a thoroughly aquatic frog. Individual members of a population are restricted in their migration by limits of water boundary. Around Delhi there are several large bodies of water which are perennial; these ponds are at a distance of 4–20 km. from each other. The climate of Delhi is dry for most of the year, and except during the rainy season (July–August) migration of frogs between ponds is not possible. During the rainy season migration may occur through a series of discontinuous puddles. At the end of the rainy season as the water dries, one

encounters many small migratory or transitory populations entrapped in small ponds. The fate of these frogs is extremely precarious; for the most part they are simply eliminated. Our collections A, B, D, and E indicated in Fig. 1, were made
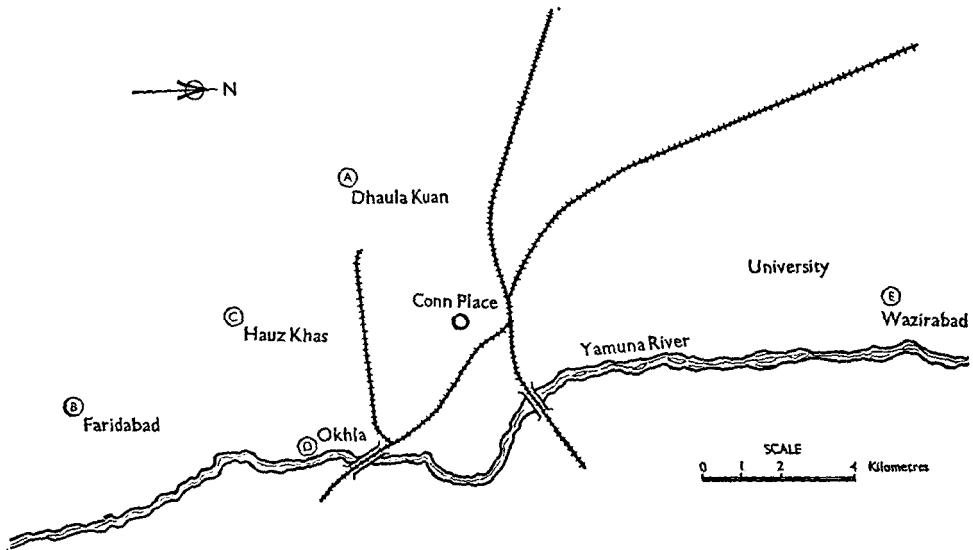

Fig. 1. Delhi map showing the places of collection of the frogs used in this study.

from large perennial water-bodies. The collection C was made from a small annual pond, and hence presumed to be a transitory population. Only adult specimens, males (10–15 g.) and females (20–35 g.), were used for this study. Their skeletons were prepared by the papain maceration technique.

3. DESCRIPTION OF SKELETAL VARIANTS

1. *Accessory ophthalmic foramen.*

The ophthalmic nerve pierces the sphenethmoid bone laterally, thus forming a single foramen on the bone. Variation is noted where the nerve branches and gives rise to more than one foramen. This may occur unilaterally or bilaterally, (Fig. 2A).

Fig. 2. A. Semi-diagrammatic representation of the skull of a frog (dorso-lateral): (1) ophthalmic foramen; (2) acessory ophthalmic foramen; (3) serrated edge of fronto-parietal; (4) smooth edge of fronto-parietal; (5) fused fronto-parietals; (6) fronto-parietal foramen; (7) oculo-motor foramen; (8) additional oculo-motor foramen. B. Semi-diagrammatic representation of maxilla: (9) maxillary foramen; (10) additional maxillary foramen. C. Simple fusion between atlas and Th-I (dorsal view): (11) notched anterior surface of atlas. D. Dorsal view of Th-II: (12) rib arch; (13) foramen at the base of rib arch. E. Ventral view of Th-VII and sacral: (14) procelous centrum of Th-VII; (15) procelous centrum of sacral. F. Diagram showing a complex fusion and the exchange of ribs between Th-VI, Th-VII and sacral (dorsal view); (16) abnormal perforation of the urostyle. G. Dorsal view of the Th-VII and sacral: (17) partially procelous centrum of Th-VII and sacral; (18) sacralization of Th-VII.
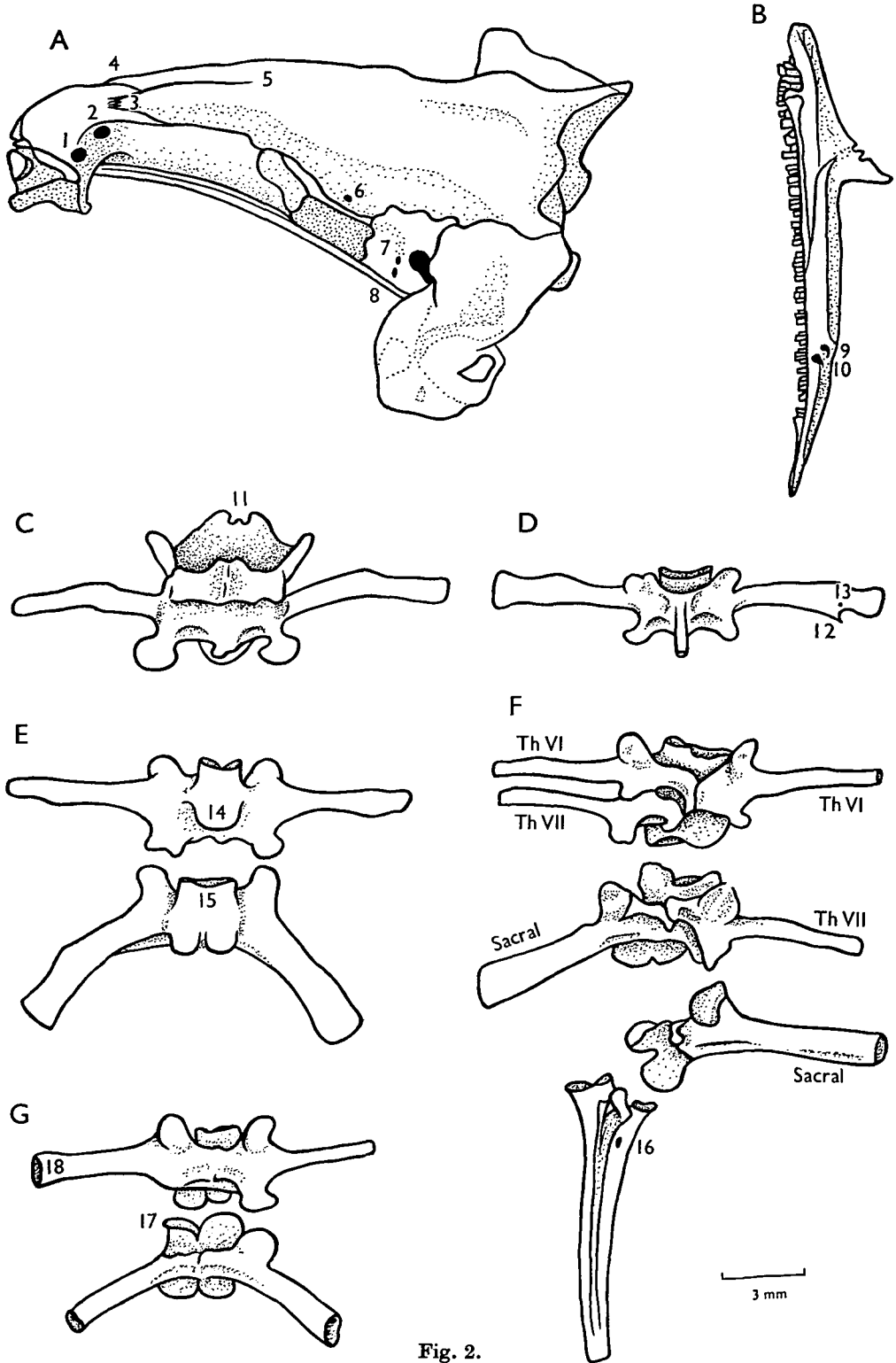
Fig. 2.

2. *Anterior edge of fronto-parietal serrated*

The anterior margins of the fronto-parietal bone may be either smooth or serrated. The character may occur unilaterally or bilaterally (Fig. 2A).

3. *Fused fronto-parietals*

When the two frontal parts of this bone are fused together throughout their length, the character is scored as fused fronto-parietal.

4. *Fronto-parietal foramen*

On the lateral sides of the fronto-parietal a foramen may be present. The occurrence of the foramen on one side of the bone is independent of its occurrence on the other side (Fig. 2A).

5. *Imperfect fronto-pariental foramen*

The fronto-parietal foramen is incompletely surrounded by bone (notch).

6. *Oculo-motor foramen double.*

Piercing the pro-otic bone is the oculo-motor nerve foramen. This foramen may be unilaterally or bilaterally double (Fig. 2A).

7. *Additional maxillary foramen*

The maxillary nerve enters the maxilla at its proximal end and leaves again through the middle of the bone. The exit point has a single or multiple foramen (Fig. 2B).

8. *Dyssymphysis of atlas*

The failure of the union of neural arches at the top.

9. *Absence of notch on the anterior surface of atlas*

Between the two articular surfaces (for the occipital condyles) lies, at the tip of the centrum, a groove of varying depth. This may be absent in certain specimens, thus giving the centrum a smooth surface on its anterior tip.

10. *Dyssymphysis of Th-I*

The failure of the union of neural arches at the top.

11. *Rib-arch of Th-II*

The transverse processes may bear an arch (spine)-like projection. This character is scored as presence or absence (Fig. 2D).

12. *Rib-arch foramen of Th-II*

The rib-arch of the transverse processes has a large vascular foramen at its base (Fig. 2D). This character is scored by unilateral presence, bilateral presence or complete absence of the foramina. The foramen is sometimes accompanied by another smaller one (Fig. 2D).

13. *Serrations on transverse processes*

The transverse processes of all or a few thoracic vertebrae may have spine-like projections. These serrations are situated on the anterior surface of the transverse processes.

14. *Exchange of transverse processes*

The occurrence of unilateral exchange of transverse processes between adjacent vertebrae (Fig. 2F).

15. *Sacralization of Th-VII*

The transverse processes of Th-VII are normally round and thin. The variant rib is broad and flattened like the sacrum, and takes part in articulation with the ilium (Fig. 2G). This occurs unilaterally.

16. *Procelous centrum of Th-VII and sacral*

The Th-VII is normally amphicelous and the sacral is acelous. In variant specimens both these vertebrae are procelous as the rest of the trunk vertebrae (Fig. 2E).

17. *Partial procelous centrum of Th-VII and sacral*

The centrum of both Th-VII and sacral are partly procelous (Fig. 2G).

18. *Vertebral fusions*

(a) *Simple vertebral fusions*. Various kinds of vertebral fusions may take place involving two or more vertebrae. The fusion between two adjacent vertebrae may either involve the articular surfaces alone (pre- and post-zygapophysis and centrum) or it may involve the neural arches as well. In some extreme cases the ribs are also fused. The structural entity of each fused vertebra remains discrete. One such simple vertebral fusion between atlas and Th-I is shown in Fig. 2C).

(b) *Vertebral fusions involving structural alterations*. The structural alterations involve one part of a vertebra being shifted on to another vertebra. One such vertebral fusion is shown in Fig. 2F. In this animal the left half of Th-VII is fused to the previous vertebra, i.e. Th-VI. The right half of Th-VII has shifted on to the sacral, thus pushing in turn the right half of the sacral vertebra out of its usual place, so that it now forms a separate entity.

19. *Perforated urostyle*

A large perforation as indicated in Fig. 2F may be present unilaterally or bilaterally.

4. RESULTS AND ANALYSIS

The percentage incidence of nineteen skeletal characters of the five populations is given in Table 1. The frequency of bilateral characters are given by the sides of the animal rather than the animals themselves. In certain cases where the specimen

was damaged or partly lost, the percentages are based on the lower total than that recorded for the population. As there was no significant difference between males and females their percentages were pooled.

Table 1. *Percentage frequency of nineteen skeletal variants in five populations of Delhi frogs*

		Number of animals				
		A	B	C	D	E
	Males	50	26	0	6	46
Variant	Females	50	46	20	54	50
1. Accessory ophthalmic foramen		22·0	15·4	10·0	9·8	12·4
2. Anterior surface of fronto-parietal serrated		57·0	55·6	90·0	51·7	40·0
3. Fused fronto-parietals		17·0	21·1	0·0	1·7	16·7
4. Fronto-parietal foramen		13·5	12·5	7·5	10·0	10·2
5. Imperfect fronto-parietal foramen		20·5	22·0	20·0	11·7	18·5
6. Oculo-motor foramen double		15·0	4·2	0·0	7·0	4·1
7. Additional maxillary foramen		45·0	42·0	82·5	44·5	31·8
8. Dyssymphysis of atlas		1·0	5·6	50·0	60·0	11·5
9. Absence of notch on anterior surface of atlas		16·0	17·0	5·0	13·5	18·8
10. Dyssymphysis of Th-I		0·0	2·8	0·0	0·0	0·0
11. Rib-arch of Th-II		75·5	69·5	39·5	15·0	62·5
12. Rib-arch foramen of Th-II		48·5	66·0	77·5	53·0	50·5
13. Serrations on transverse processes		37·0	7·0	0·0	1·7	7·5
14. Exchange of ribs		5·0	1·4	5·0	3·4	2·1
15. Sacralization of Th-VII		0·0	0·0	0·0	1·7	0·0
16. Procelous centrum of Th-VII and sacral		7·0	11·1	0·0	6·8	6·3
17. Partial procelous centrum of Th-VII and sacral		1·0	2·8	0·0	5·1	2·1
18. Vertebral fusions		9·0	5·6	5·0	11·9	3·2
19. Perforated urostyle		12·0	14·0	10·0	10·0	6·3

In order to study the degree of divergence between these populations, the percentage incidences from Table 1 were transformed into angular values by C. A. B. Smith's method (see Berry, 1963) as this makes that part of the variance which is due to errors of sampling independent of the incidence of the characters. This angular value, θ, is related to the percentage incidence (p) by the formula

$$\theta = \sin^{-1}(1 - 2p)$$

measured in radians. The next step is to construct a measure of the divergence between the populations. For any two populations this is done by taking the differences between the angular values for each of the nineteen characters studied, squaring these differences, adding them together and finally dividing the sum by the number of characters (i.e. 19). This raw measure of divergence still contains a part which can be ascribed to random sampling fluctuations. This is allowed for by subtracting

$$\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$$

from the uncorrected measure of divergence between any two populations

N_1 and N_2, where n_1 and n_2 are the number of frogs in each population. This measure of divergence between any two populations shows how far, on an average, the nineteen characters have diverged from each other. Its standard error is calculated as

$$\text{S.E.} = \sqrt{\frac{4\left(\dfrac{1}{n_1}+\dfrac{1}{n_2}\right) \times \text{Measure of divergence}}{\text{Number of characters}}}$$

The divergences and their standard errors are given in Table 2.

5. DISCUSSION

Our analysis of the five populations of Delhi frogs indicates the existence of considerable inter-population diversity. The approach to show diversity is necessarily statistical. The measure of divergence between the populations, and standard errors calculated to provide the significance of this measure of divergence is shown in Table 2. An examination of this table shows that populations B and E

Table 2. *Measure of divergence of nineteen characters between five populations of Delhi frogs. Estimates of standard errors are indicated within brackets*

	B	C	D	E
A	0·042	0·351	0·293	0·057
	(0·015)	(0·067)	(0·041)	(0·016)
B		0·219	0·186	0·006
		(0·054)	(0·035)	(0·006)
C			0·107	0·232
			(0·039)	(0·054)
D				0·135
				(0·028)

are similar, and all other populations differ significantly from each other (the measure of divergence being three or more times its standard error).

A consideration of the individual characters also indicates that certain characters show a considerable degree of fixation in some of the populations. Thus a glance at Table 1 shows that 'serrations on transverse processes' has a very high frequency in population A; 'fused fronto-parietals' being absent in population C and low in population D; 'rib-arch on Th-II' being low in population D and high in population A.

The question thus resolves itself into not whether inter-population differences exist, for indeed they do, but what are the factors to which these differences can be ascribed.

Phenotypic expression of a character which shows individual variation and differentiation between several populations serves as a parameter in the study of micro-evolution. The analysis of variation, when complemented by inquiries into the genetic nature and adaptive significance of the characters chosen, proves a useful guide to the study of evolution in action.

In the present study of skeletal variants, the genetic nature of the variants is largely inferred from the work on mice (Grüneberg, 1951, 1952; Grewal, 1962b; Searle, 1960), and from Highton's (1960) findings in red-backed salamanders, where the number of vertebrae is genetically controlled. Our own work on vertebral fusions in this frog (unpublished) shows that that character is genetically controlled.

The populations A, B, D, and E were from a similar habitat, i.e. large perennial ponds. They also share the same climatic conditions of the city. In the absence of any apparent environmental differences, it is probable that this inter-population diversity is due to random drift. However, the possibility of selective forces which are at present not detectable cannot be ruled out.

SUMMARY

Nineteen axial skeletal variants are described in the frog *Rana cyanophlictis*, collected from five isolated populations of Delhi. Four of these populations came from large perennial ponds and one, a transitory population, was entrapped from a temporary rain pool. The populations were found to diverge significantly from each other in the incidence of these skeletal variants. We have attributed this divergence to genetic drift, but the effects of unknown selective forces cannot be ruled out.

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