

T ECOLOGICAL AND SYSTEMATIC RELATIONSHIPS  
IN NATAL RANIDS (RANA LINN. SENSU STRICTO AND  
SENSU STRONGYLOPUS TSCHUDI) WITH NOTES ON A  
POSSIBLE COMPETITOR, THE LEPTODACTYLID  
HELEOPHRYNE NATALENSIS HEWITT


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## 1. INTRODUCTION

### 1.1 Nomenclatural history

The genus Rana was erected by Linnaeus in 1758, and has a long history of subdivision. I will only be concerned with the history of "Rana" in southern Africa.

- 1824 Burchell describes Rana fasciata collected in southern Africa.
- pre 1838 Boie lists a specimen in the Leyden Museum from South Africa as Rana fasciata.
- 1838 Tschudi erects the genus Strongylopus on the specimen of R. fasciata Boie, separating the new genus on the basis of differences in tympanum, vomerine teeth and toes from typical Rana.
- 1841 D  meril and Bibron synonymize Strongylopus with Rana because of similarities of tympanum and vomerine teeth. They describe R. fuscigula and R. delalandii.
- 1849 Andrew Smith describes R. grayi.
- 1866 Bocage records a new species, R. angolensis.
- 1894 Boulenger describes R. quecketti from Pietermaritzburg.
- 1895 Bocage synonymizes R. delalandii with his R. angolensis.
- 1896 Boulenger describes R. nutti.
- 1903 Boulenger identifies both R. angolensis and R. delalandii from the Cape, which Bocage had synonymized.
- 1910 Boulenger synonymizes R. quecketti with R. fuscigula and R. angolensis with R. delalandii.
- 1918 Boulenger changes his mind about R. quecketti as a synonym of R. fuscigula, and synonymizes R. quecketti with R. angolensis.
- 1920 Boulenger describes R. hymenopus.
- 1927 Hewitt describes R. vertebralis from the summit of Mont aux Sources. Later in the year he recognises a hybrid R. grayi x R. vertebralis.
- 1947 FitzSimons describes Phrynobatrachus lawrencei.
- 1948 FitzSimons describes R. draconensis.
- 1952 Bush describes R. umbraculata from the Umzimkulu river.
- 1961 Wager inadvertently describes R. wageri.
- 1962 Parker and Ride show that no type exists for R. fasciata Burchell, and that consequently R. grayi and R. fasciata have been confused. They request the International Commission of Zoological Nomenclature to designate neotypes.



- 1963 H.M. Smith requests the Commission to cite Andrew Smith as author of R.fasciata as good illustrations accompany A.Smith's descriptions of R.fasciata and R.grayi.
- 1963 Poynton formally describes R.wageri Wager.
- 1964 Poynton publishes the first comprehensive revision of the amphibia of Southern Africa. He synonymizes R.delalandii D&B, R.nutti Boul., R.theileri Mocquard with R.angolensis Bocage. Also R. quecketti Boul. with R. fuscigula D&B; R. umbraculata Bush with R. vertebralis Hewitt; R. draconensis FitzSimons, R. grayi x R. vertebralis Hewitt and Phrynobatrachus lawrencei FitzSimons with R. hymenopus Boul. Poynton notes that only R. angolensis, R. fuscigula and R. vertebralis have typical omosterna, while grayi, fasciata, hymenopus and wageri have forked omosterna. He writes "on the basis of this Strongylopus Tschudi could be revived as a subgenus to accomodate these latter forms" (p 92). Poynton, however, only formally recognizes the genus Rana.
- 1965 Wager only recognises Rana.
- 1966 Van Dijk separates Rana from Strongylopus on the basis of tadpole morphology. He recognises R. angolensis, R. fuscigula, R. vertebralis and S. grayi, S. fasciatus, S. wageri and S. hymenopus.
- 1976 Carruthers only recognises Rana.
- 1976 Channing and Van Dijk recognise Strongylopus and Rana.
- 1976 Pienaar, Passmore and Carruthers recognise Rana only.

At the commencement of this study seven species were generally recognised, of which four were placed either into the genus Rana or the genus Strongylopus. As the results of this study have a bearing on the generic status of the species studied, I have referred to them throughout the thesis by their specific names only, avoiding the use of "Rana" or "Strongylopus" so as not to pre-empt the conclusion.

## 1.2 Aim of this study

At the commencement of this study I intended to investigate the biology of the Rana/Strongylopus group of frogs, and to compare their ecology with that of the only other "river" frog in the area utilizing the banks along streams, Heleophryne natalensis. I hoped to be able to explain the selection pressures leading to the evolution of the present group and to elucidate the

isolating mechanisms and special adaptations of each species. I was especially interested in R. angolensis, which appeared to be widely distributed in almost all habitats from the top of the Drakensberg to the coastal plain.

### 1.3 Brief description of the study area

The area of the study (fig. 1) was selected because all the species in the group occur within it and it was easily accessible from Pietermaritzburg. The Drakensberg plateau was within the range of a four-wheel-drive vehicle up Sani Pass.

Southern Africa's highest point (Ntabantleyana 3385m) occurs just north of the area. Topographically a wide range of landforms are included, from the Lesotho plateau at ca. 3000m, the Drakensberg escarpment, the Natal uplands and midlands, and the narrow coastal belt at Durban. The vegetation of the area varies from alpine on the Drakensberg, to grassland and coastal bush. Mist forest and the remnants of a wider forest belt are found on steep slopes in the Karkloof near Howick and in patches over most of the area.

Geologically the area includes Drakensberg basalt, sedimentary rocks of the Stormberg, Beaufort and Ecca series, Dwyka tillite and Table Mountain sandstones overlaying the basement granites and gneisses.

Rainfall decreases westward from over 1000mm at the coast to 635mm on the Drakensberg (fig. 1). Temperatures vary from 25,3°C average daily maximum to 4,5°C average daily minimum.

Fuller descriptions of the area's vegetation may be found in Killick (1963) and Acocks (1975) while King (1972) explains the geomorphology. Climatic data are available from the relevant Weather Bureau publications.

### 1.4 Outline of approach

The species studied (angolensis, fuscigula, vertebralis, grayi, fasciata, hymenopus, wageri and "D"(see 1.5)) were examined for morphology, colour pattern morphs, plasma proteins, oxygen dissociation curves of blood, mating call, general ecology and distribution. Although Poynton recognised subspecies of fasciata and grayi (1964a), only the nominate subspecies occur within the study area. As many sites as possible were examined to determine the ecological requirements of each species. A few sites just outside the study area were visited to collect uncommon species.



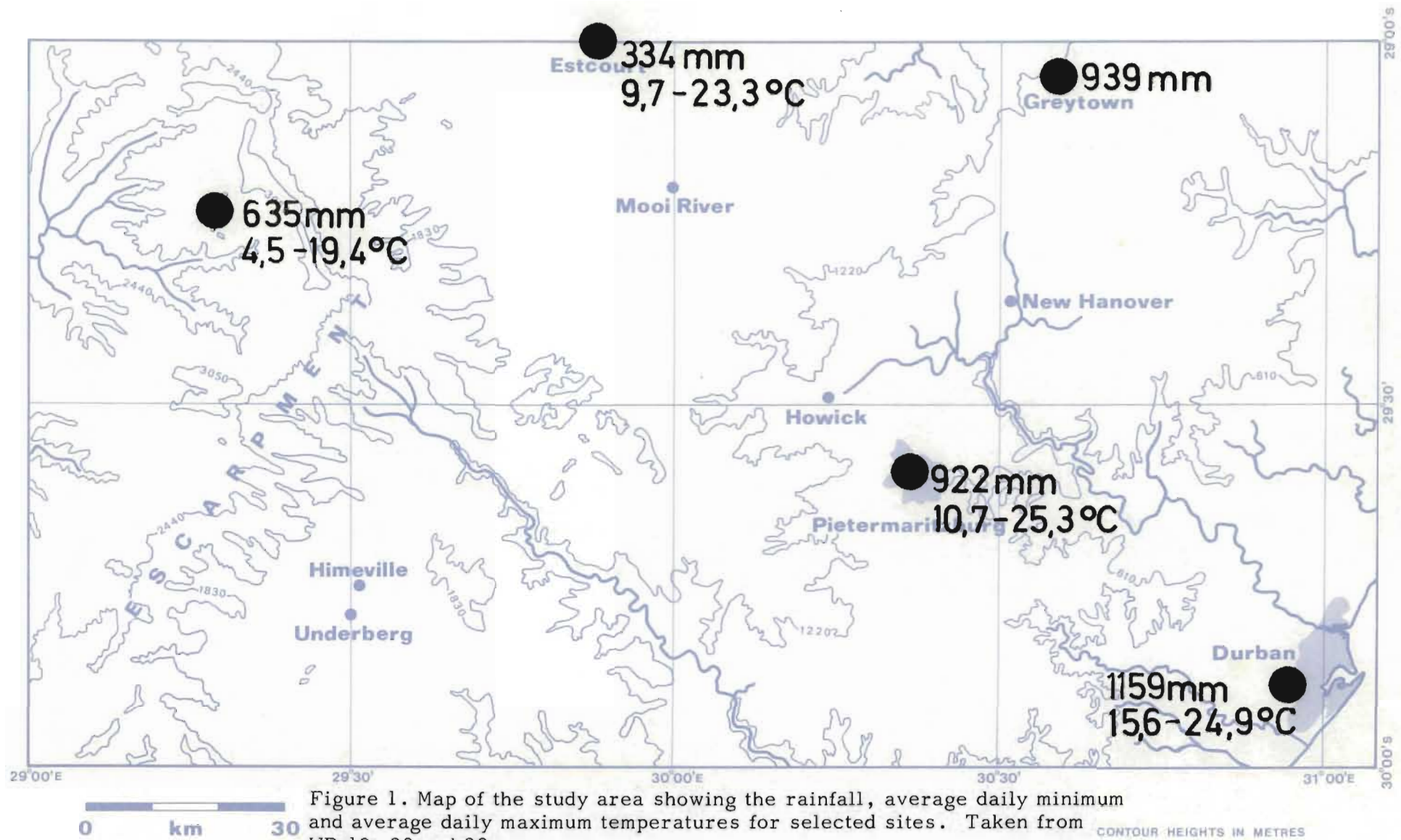


Figure 1. Map of the study area showing the rainfall, average daily minimum and average daily maximum temperatures for selected sites. Taken from WB 19, 20 and 28.



At least three of the species (vertebralis, wageri and hymenopus) are uncommon and were collected inside and outside the study area proper.

Due to the practical problems of personally examining habitats over a large area, my approach has been to concentrate on a relatively limited area and to visit each site as frequently as possible throughout the year.

### 1.5 Recognition of a new species

At the commencement of this study I experienced some difficulty in identifying one of the species from the Drakensberg. At first glance in the field the frogs were identified as fuscigula, yet on keying them out they appeared to be angolensis. Later it became apparent that the oxygen capacity of this form differed from typical angolensis. Natal angolensis from sea level to 2344m possessed the same dissociation curve, while the Drakensberg population only about 500m above a typical angolensis population possessed a dissociation curve displaced to the left. It seemed unlikely that the blood carrying capacity of angolensis would remain unchanged through 2344m, only responding to the last 500m, if a simple phenotypic difference were being measured. During the final stages of this study it became obvious that this form differed in all respects except colour from typical angolensis. Even the mating call is different, indicating reproductive isolation. I have treated this form as a distinct species, and referred to it as "D" throughout this thesis.

### 1.6 The study species

Maps of the distributions of the species studied are given in figure 2a, based on Van Dijk (1977). The following maximum lengths have been recorded (Poynton 1964a): angolensis 81 mm, fuscigula 123 mm, vertebralis 140 mm, wageri 46 mm, grayi 50 mm (as grayi grayi), fasciata 50 mm (as fasciata fasciata), hymenopus 65 mm. The largest "D" found has a length of 65 mm. Photographs of all the species except "D" are presented in figure 2b.

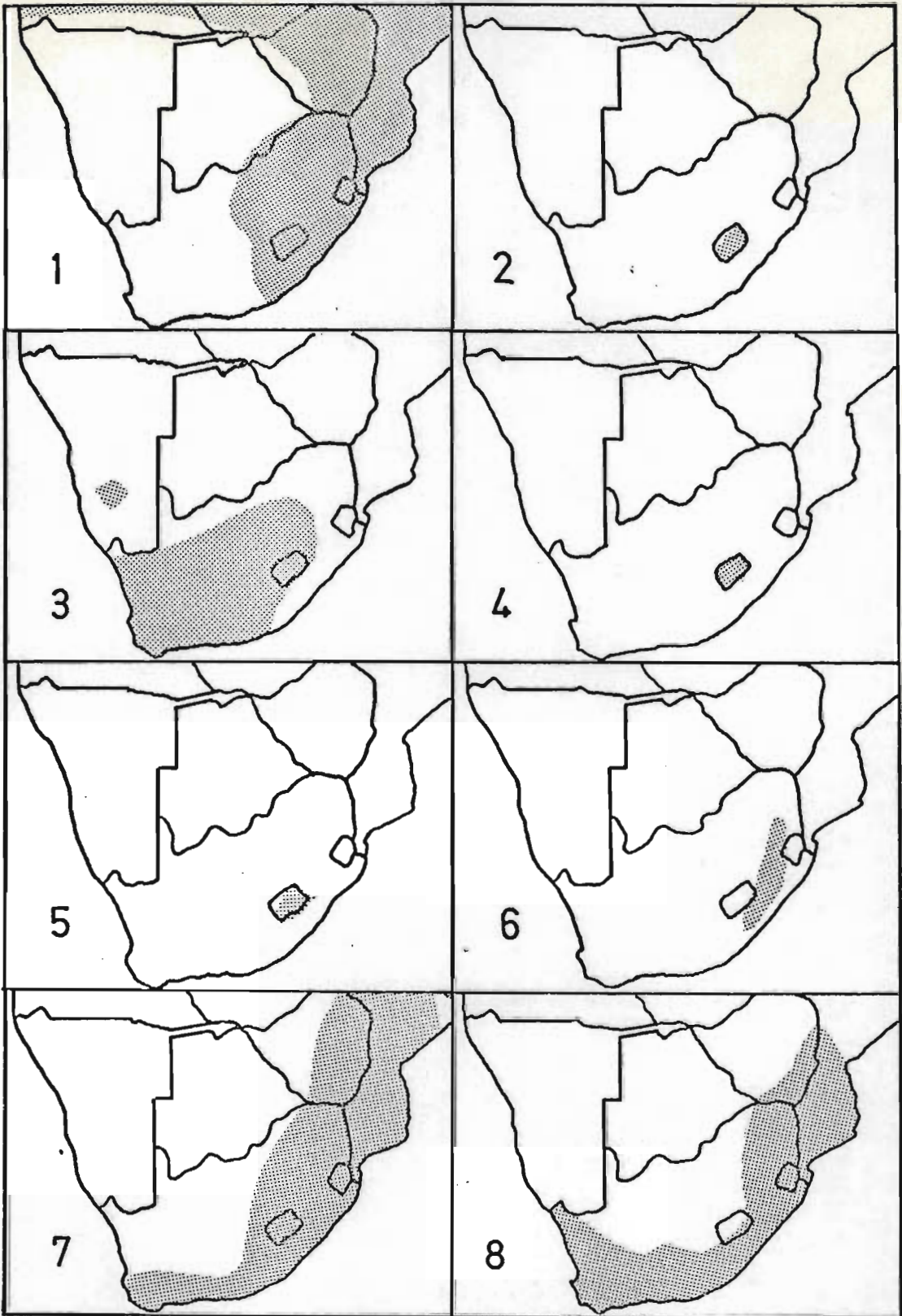


Figure 2a. Distributions of the species studied: *angolensis* 1, "D" 2, *fuscigula* 3, *vertebralis* 4, *hymenopus* 5, *wageri* 6, *fasciata* 7, *grayi* 8. Maps of southern Africa, including South West Africa, Botswana and Rhodesia.

Figure 2b. Photographs of the species studied.

1. vertebralis
2. Anterior view of vertebralis to show the umbracula in the eyes
3. fuscigula
4. angolensis
5. wageri
6. hymenopus
7. fasciata
8. grayi



1



2



3



4



5



6



7



8





## 2 OXYGEN DISSOCIATION CURVES OF WHOLE BLOOD

Dissociation curves of various vertebrates exhibit different relative positions and shapes. Aquatic forms load the haemoglobin at lower oxygen pressures (lower half saturation pressure ( $P_{50}$ )) than terrestrial forms. Such a "left-handed" curve allows extraction of oxygen from an environment poor in oxygen relative to air. Mammals that are native to high altitudes also have dissociation curves to the left of their low-altitude relations (Gordon 1972). This study was aimed at determining the effect of altitude and the aquatic/terrestrial way of life on the dissociation curves of the species studied.

### 2.1 Methods

The following is an outline of the methods used. Full details are given in the appendix. Blood was removed from frogs which had been anaesthetized in a 1% solution of Benzocaine. The heart was exposed by dissection and blood was drawn off using a micro-haematocrit tube and collected in a watch glass moistened with a 3% solution of sodium oxalate to prevent clotting. The watch glass was covered and the blood stirred with a magnetic stirrer while the dissociation curve was determined on small aliquots.

The technique for dissociation curve determination was derived from that used by Sun and Helman (1974). It depends on spectrophotometric measurement of oxygen saturation based on the relationship between the absorption spectra of oxidised and reduced haemoglobin. There are basically four steps:

- 1) Preparation of 100% and 0% oxygen saturation standards.
- 2) Determination of the two wavelengths at which the sample is to be measured and construction of a calibration curve.
- 3) Equilibration of the blood at various partial pressures of oxygen.
- 4) Preparation of the sample for the spectrophotometer, and measurement of the percentage saturation.

Steps 1 and 2 above are necessary for each new species studied, but once a satisfactory curve is obtained it is only necessary to repeat steps 3 and 4 for each subsequent frog examined of a 'known' species. The dissociation curve is plotted as percent saturation of blood against partial pressure of oxygen. The curves were all determined at 20°C, at physiological pH and using whole blood. The results for each sample were

pooled and averaged, and fitted to a log. curve. Linear transformations of these curves were plotted to determine the sigmoid coefficient ( $n$ ) of the Hill equation. This involves plotting  $\log_e (y/100-y)$  against  $\log_e P_{O_2}$  ( $y$ =%saturation). Regressions used are in the appendix. When possible the erythrocytes were counted, the haematocrit, and the concentration of haemoglobin (as g/100ml) were determined. Erythrocyte counts were carried out after the blood had been diluted 1:100 in a diluent described by Quay (1974). The haematocrit was obtained by spinning the sample in micro-haematocrit tubes in a clinical centrifuge for ten minutes. Haemoglobin concentrations were determined spectrophotometrically using a 'Merck' haemoglobin test kit.

## 2.2 Results

The distribution of the localities from which frogs were collected for equilibrium curve (dissociation curve) determinations is given in figure 3. The equilibrium curves are presented in figure 4. (Note that the coefficient of determination ( $r^2$ ) is a measure of the goodness-of-fit of the data. A perfect fit would have  $r^2=1,00$ .) Seven of the eight curves are logarithmic, the data fitting very closely to the curves ( $r^2=0,97-0,98$ ). The curve for hymenopus fitted a straight line (method of least squares,  $r^2=0,99$ ). The linear transformations of the curves are given in figure 5. The slope of the line =  $n$  (sigmoid coefficient of the Hill equilibrium equation). The following values for  $n$  were obtained: "D", fasciata and fuscigula - 1,67; angolensis - 1,25; wageri - 1,1; vertebralis - 0,96; hymenopus - 0,84; grayi - 0,74.

For comparative purposes the pressure at which blood is half saturated ( $P_{50}$ ) is regarded as the useful unloading pressure (Gordon 1972) and it is this pressure that I have used to illustrate the abilities of the different species to live under various oxygen tensions in aquatic and terrestrial environments. The haematocrits, erythrocyte counts, haemoglobin concentrations,  $P_{50}$  values and blood pH are compared in figure 6.

## 2.3 Correlation of $P_{50}$ with other blood parameters

The  $P_{50}$  of each species was correlated with haematocrit, haemoglobin, erythrocyte count and extent of webbing (fig. 23). Using the Kendall coefficient of concordance ( $W$ ) the null hypothesis "that there is no correlation" was tested.  $W$  was significant at the 0,02 level (i.e. the null hypothesis was rejected) which suggests strongly that when a frog in this group adapts to an aquatic or terrestrial way of life (or any intermediate



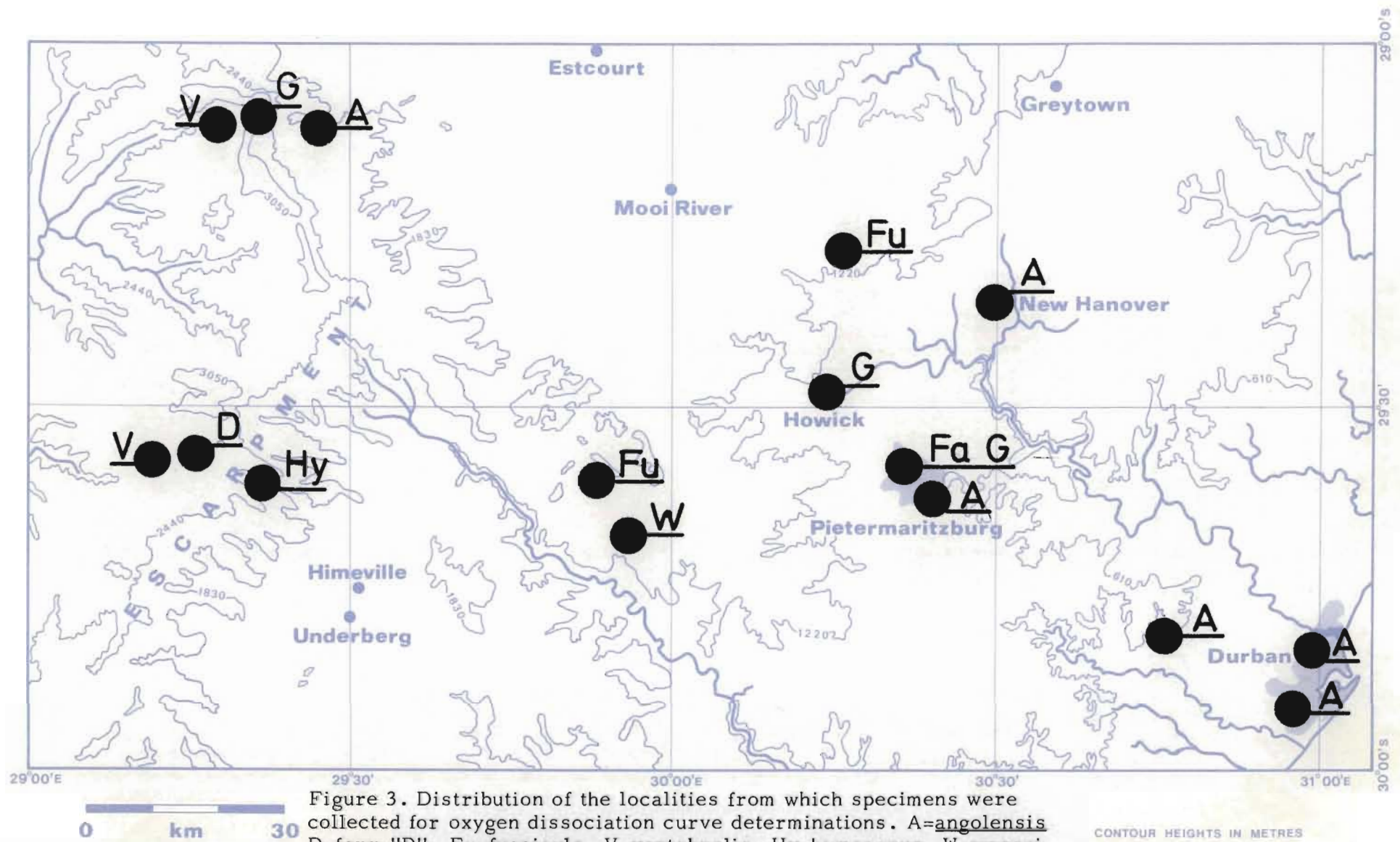


Figure 3. Distribution of the localities from which specimens were collected for oxygen dissociation curve determinations. A=*angolensis*, D=form "D", Fu=*fuscigula*, V=*vertebralis*, Hy=*hymenopus*, W=*wageri*, Fa=*fasciata*, G=*grayi*.

CONTOUR HEIGHTS IN METRES

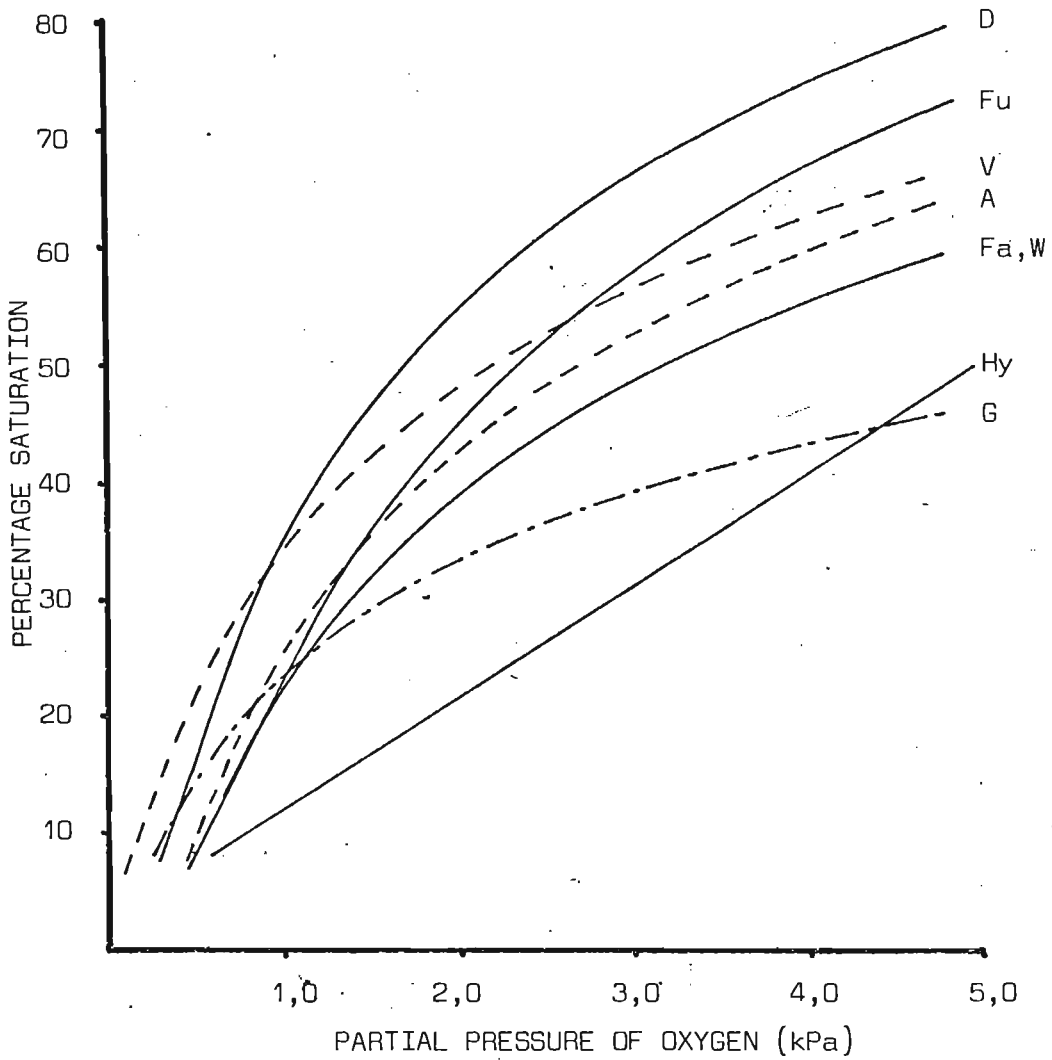


Figure 4. Dissociation curves of whole frog blood. Abbreviations of species as in figure 3. Correlation coefficients and half saturation pressures as follows (all curves determined at 20°C and physiological pH (fig.6):

angolensis  $r^2=0,98$ ;  $P_{50}=2,7$  kPa

form "D"  $r^2=0,98$ ;  $P_{50}=1,7$  kPa

fuscigula  $r^2=0,98$ ;  $P_{50}=2,4$  kPa

vertebralis  $r^2=0,98$ ;  $P_{50}=2,1$  kPa

hymenopus  $r^2=0,99$ ;  $P_{50}=5,0$  kPa

wageri  $r^2=0,98$ ;  $P_{50}=3,1$  kPa

fasciata  $r^2=0,98$ ;  $P_{50}=3,2$  kPa

grayi  $r^2=0,97$ ;  $P_{50}=6,3$  kPa



point), this specialization is reflected in the blood parameters and the amount of webbing.

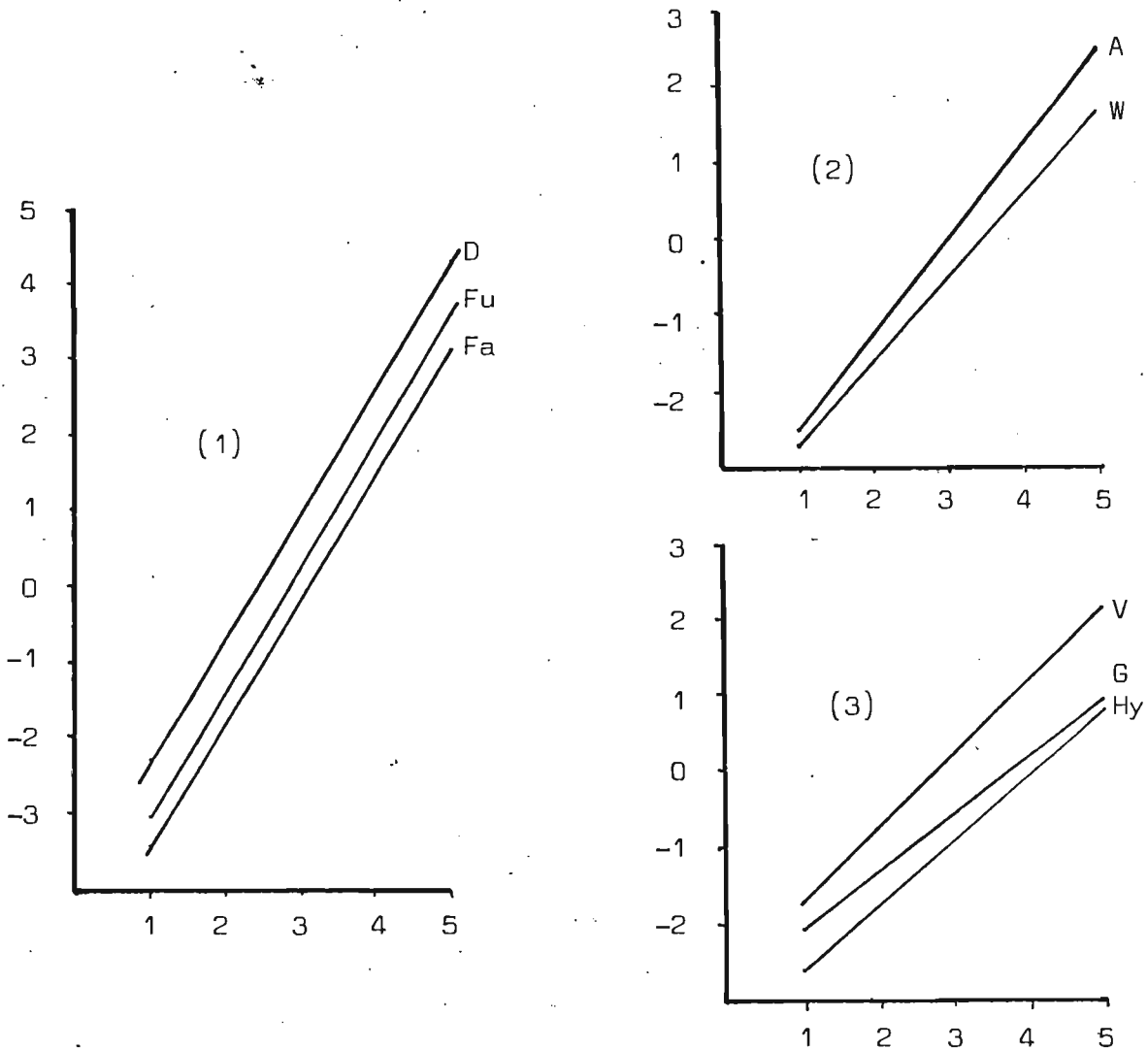


Figure 5. Dissociation curves transformed to find (n) using the Hill Equation. Ordinate is  $\log_e (y/100-y)$ , abscissa is  $\log_e P_{O_2}$  (mmHg).  $y$ =% saturation. Slope of each line is (n). Abbreviations for species as in fig. 3. (1)= slopes for "D", fuscigula and fasciata; (2)= slopes for angolensis and wageri; (3)= slopes for vertebralis, grayi and hymenopus.

## 2.4 Discussion

Previous studies on the number of erythrocytes in Rana from North America give values of 170 000 – 390 000mm<sup>3</sup> for R. catesbeiana and 135 000 – 460 000mm<sup>3</sup> for R. clamitans (Hutchinson and Szarski 1965). Rouf (1969) provides a summary of blood studies on European and United States Rana, which have erythrocyte counts ranging from 166 000 to 800 000. He found that R. pipiens has an erythrocyte count of 120 000 to 470 000 ( $\bar{x}$  319 400), pH 7,2 – 7,6 ( $\bar{x}$  7,36), haematocrit of 13 – 39% ( $\bar{x}$  24,65) and haemoglobin concentration of 2,4 – 9,6 g/100ml ( $\bar{x}$  6,75). These are comparable to my results summarized in fig. 6, except that hymenopus and fasciata have slightly higher erythrocyte counts.

Previously published records for half saturation values of whole frog blood vary between 13,2mmHg to 39,1mmHg (1,8 – 5,2kPa) for Rana (Prosser 1973). These agree well with my results (2,0 – 6,3 kPa).

From the dissociation curves it can be seen that vertebralis and "D" are most aquatic and/or adapted for high altitude, while grayi and hymenopus are most terrestrial and/or adapted to low altitudes. As hymenopus only occurs at relatively high altitude and grayi is frequently found at 3000m, it seems that altitude is not a factor influencing the position of the curve in these animals, probably because 3000m is too low to require any increased O<sub>2</sub> uptake to compensate for decreased P<sub>O<sub>2</sub></sub> due to altitude. Gordon (1972) shows that alveolar P<sub>O<sub>2</sub></sub> drops by only a few percent from sea level to 3000m. On the other hand, the dissociation curves were determined at 20°C, but the curves will shift left with a decrease in temperature (Prosser 1973). This suggests that the species do not require a left shifted curve for altitude per se, as the reduced temperatures of the high mountains will cause the curve to shift left and result in the availability of more oxygen to the tissues.

Packard (1972) showed that toads are adapted for optimum O<sub>2</sub> consumption at the environmental temperatures under which they live. This supports the view that the water/air environment is primarily associated with the position of the dissociation curve in these animals, as the altitude differences are minimal, and they are probably adapted to the temperatures of their individual habitats. The terrestrial habits of grayi and hymenopus agree well with the positions of their dissociation curves.

Diphosphoglycerate (DPG) is known to cause a right-shift in whole blood dissociation curves (Gordon 1972). The position of the curve may be dependant on the level of DPG in the blood. This would permit

adaptations in the position of the curve to depend on modifications in DPG level.

The shape of the dissociation curve of whole frog blood is described as sigmoid. When the sigmoid coefficient is low, as is the case in these frogs, the curve becomes hyperbolic (Jones 1972). Few species are known with sigmoid coefficients below 1 (Jones 1972), partly because few anuran species have been studied to date (Prosser 1973). Flat curves like that of hymenopus and grayi with low  $n$  values may prove to be more common in the group as a whole. Squalus suckleyi (spiny dogfish) and Eupolymnia sp. (polychaete worm) are known to have low sigmoid coefficients (Jones 1972). The value of  $n$  is apparently related to the chemistry of the haem-haem interactions during equilibration, although the actual mechanisms involved are unknown. A study of these mechanisms would be most rewarding, but is beyond the scope of this investigation.

	Haematocrit (%volume)	Erythrocyte count/mm <sup>3</sup>	Haemoglobin (g/100ml)	P <sub>50</sub> (kPa)	pH	n
V	23,7 (21-25)	7,0×10 <sup>5</sup>	6,0 (4,4-7,0)	2,1	7,4	23
Fu	23,6 (14-34)	5,3×10 <sup>5</sup>	6,5 (4,7-7,7)	2,4	7,5	38
D	22,8 (20-26)	9,1×10 <sup>5</sup>	6,0 (4,7-7,0)	1,7	7,5	14
A	24,1 (14-40)	9,1×10 <sup>5</sup>	6,0 (2,2-10,4)	2,7	7,5	56
Hy	29,5 (27-32)	1,1×10 <sup>6</sup>	9,9	5,0	7,4	5
W	29,5 (23-32)	6,0×10 <sup>5</sup>	8,2	3,1	7,3	9
Fa	23,2 (10-40)	1,2×10 <sup>6</sup>	6,3 (1,8-10,2)	3,2	7,3	60
G	23,1 (18-27)	9,7×10 <sup>5</sup>	6,1	6,3	7,5	52

Figure 6. Table of mean values for haematocrit, erythrocyte count, concentration of haemoglobin, half saturation values and blood pH. Range in brackets. Abbreviations: V=vertebralis, Fu=fuscigula, D= form "D", A=angolensis Hy=hymenopus, W=wageri, Fa=fasciata, G=grayi.

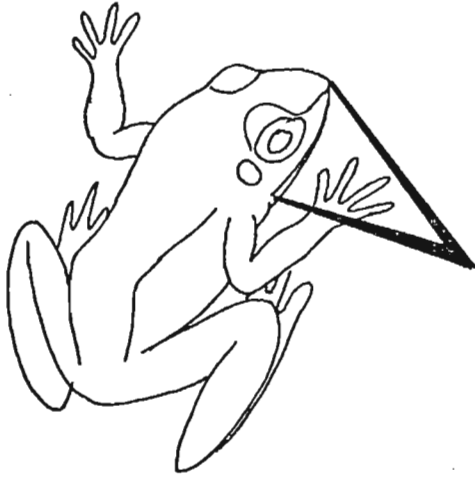


Figure 7. Illustration of the measurement of angle of jaw to tip of snout.



### 3 MORPHOLOGY

#### 3.1 Mensuration

The following measurements were taken (fig.7):

1. Snout-urostyle length, with the animal pressed flat.
2. Width of head at widest point.
3. Head height, measured from the lower jaw to the top of the skull at the level of the tympanum.
4. Head length, measured from the angle of the jaw to the tip of the snout.
5. Horizontal diameter of eye.
6. Horizontal diameter of tympanum.
7. Distance between outside edges of ilia at level of the sacrum.
8. Sacro-urostyle length measured along the right ilium.
9. The number of phalanges free of webbing on both sides of the fourth toes.
10. Tibia length, measured on the folded right tibia.
11. Length of foot including fourth toe.
12. Length of radio-ulna measured on the folded right forearm
13. Length of hand measured from the inner metacarpal tubercle to the tip of the longest finger.

All measurements were taken on the animal's right. If some part of the right side was damaged, all measurements were then taken on the left. The distribution of localities from which specimens were examined is shown in figure 8.

#### 3.2 Selection of ratios

To emphasize similarities and differences between species the following ratios were used:

$$\begin{array}{cccccc} \frac{\text{head width}}{\text{snout-urostyle}} & \frac{\text{tympanum}}{\text{head width}} & \frac{\text{tympanum}}{\text{eye}} & \frac{\text{head length}}{\text{head width}} & \frac{\text{head width}}{\text{tibia}} & \frac{\text{foot}}{\text{head width}} \\ \frac{\text{ilia width}}{\text{sacrum-urostyle}} & \frac{\text{tibia}}{\text{snout-urostyle}} & \frac{\text{ulna}}{\text{toe}} & \frac{\text{head height}}{\text{head width}} & \frac{\text{hand length}}{\text{head width}} & . \end{array}$$

The extent of webbing was investigated because this character is commonly used in identification and classification, yet no studies on the variation of webbing in southern African rana have been published. The degree of webbing is a useful indicator of the terrestrial/aquatic nature of each species, as completely aquatic species are usually fully webbed, while more terrestrial species are progressively less webbed. (See also 2.3)



### 3.3 Results

The graphs comparing the ratios of the morphological proportions are presented in figs 10 – 20. Illustrations of the ventral surfaces of the feet are shown in figs 21 and 22. Fig. 23 gives webbing frequencies.

Difference indices were calculated for each species pair for all the ratios, using the formula of Handford and Nottebohn (1976):

$$D_m = \frac{1}{n} \sum_{i=1}^n \left| \frac{\bar{x}_{ij} - \bar{x}_{ik}}{\bar{x}_{ij} + \bar{x}_{ik}} \right|$$

where  $n$  = number of characters measured,  $\bar{x}_{ij}$  is the mean value of character  $i$  in sample  $j$ , and  $\bar{x}_{ik}$  is the mean value of character  $i$  in sample  $k$ . The morphological differences indices are given in figure 9.

MORPHOLOGICAL DIFFERENCE INDICES ( $D_m$ )

	G	Fa	W	Hy	A	D	Fu
V	0,14	0,21	0,15	0,08	0,17	0,13	0,10
Fu	0,09	0,17	0,65	0,08	0,08	0,04	
D	0,05	0,14	0,08	0,06	0,05		
A	0,04	0,09	0,05	0,09			
Hy	0,06	0,13	0,07				
W	0,04	0,06					
Fa	0,08						

Figure 9. Morphological difference indices calculated for all species pairs using mean ratios. G=grayi, Fa=fasciata, W=wageri, Hy=hymenopus, A=angolensis, D=form "D", Fu=fuscigula, V=vertebralis.

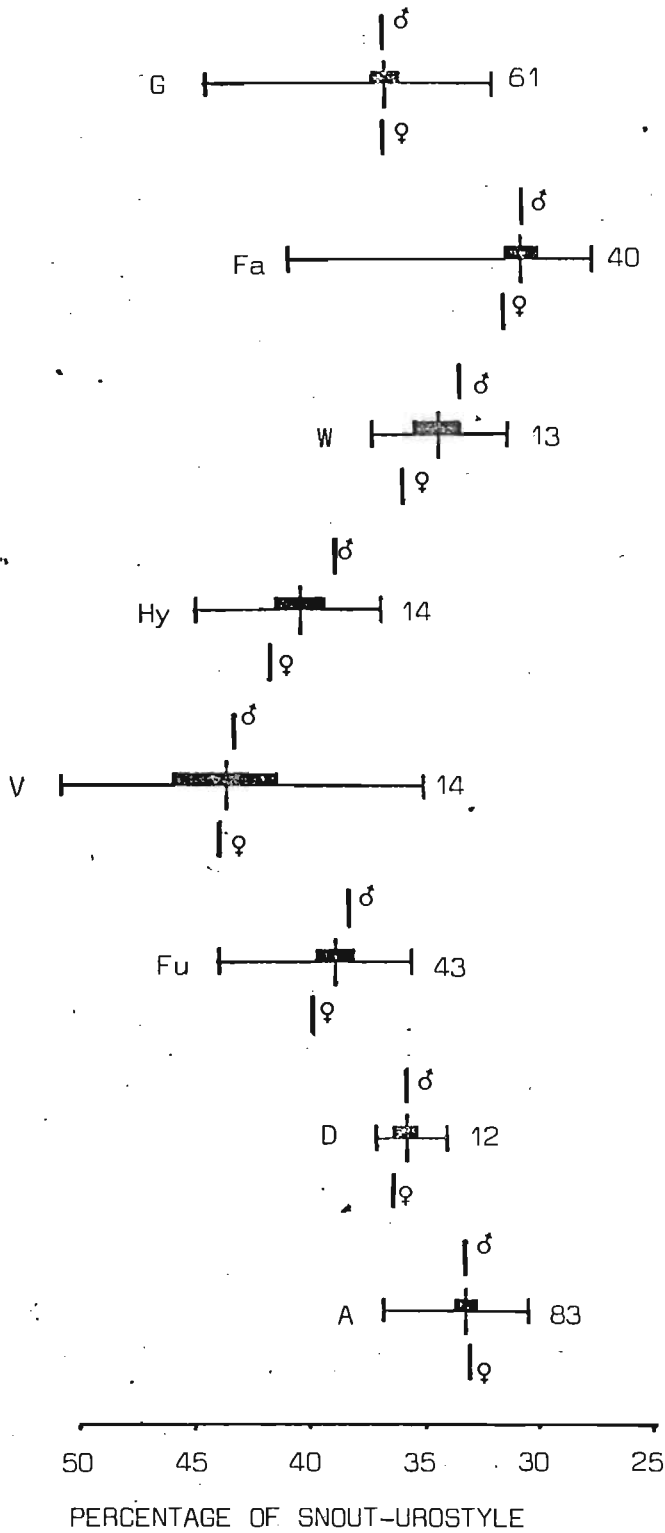


Figure 10. Ratios of head width/snout-urostyle. Abbreviations of species as in figure 9. Number to right of bar is sample size. Horizontal line shows range, broad solid block is mean  $\pm$  2 standard errors. Vertical line is mean. Male and female means shown above and below range line, respectively.



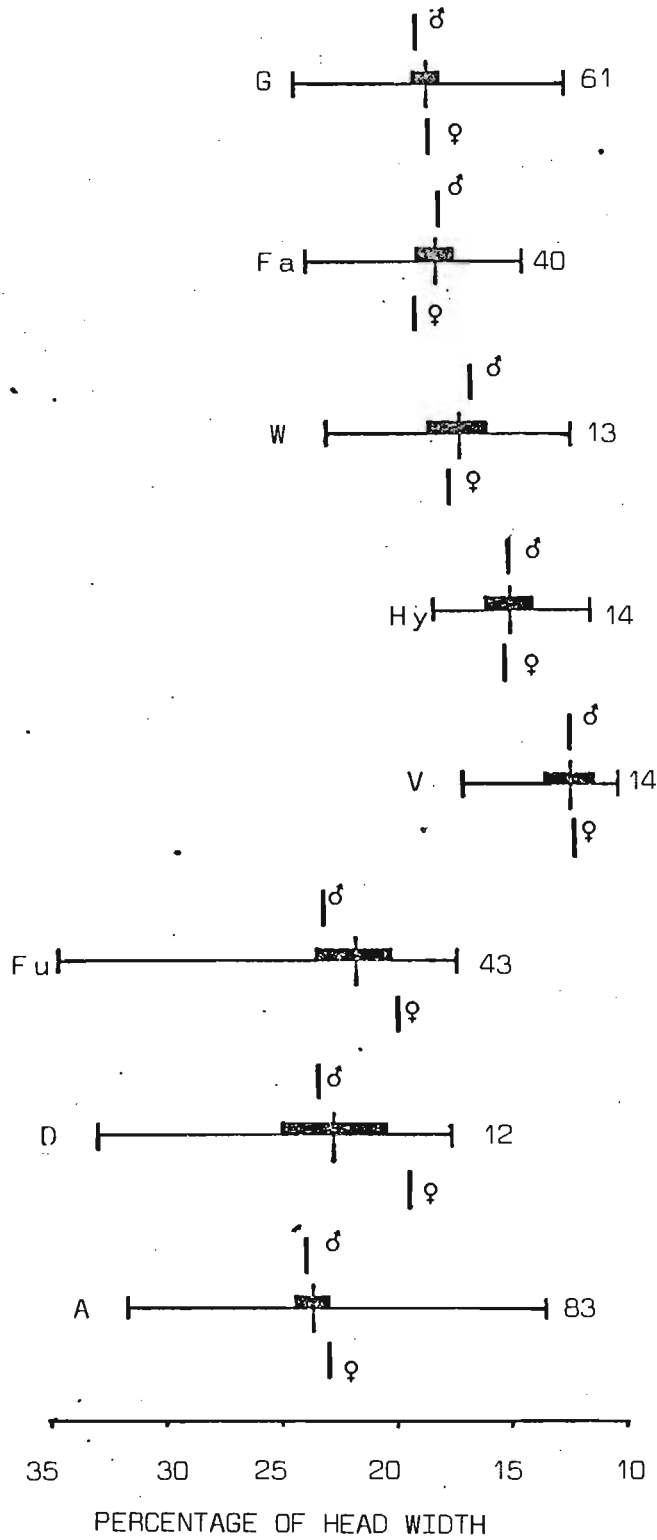


Figure 11. Ratios of tympanum/head width. For explanation see fig. 10.

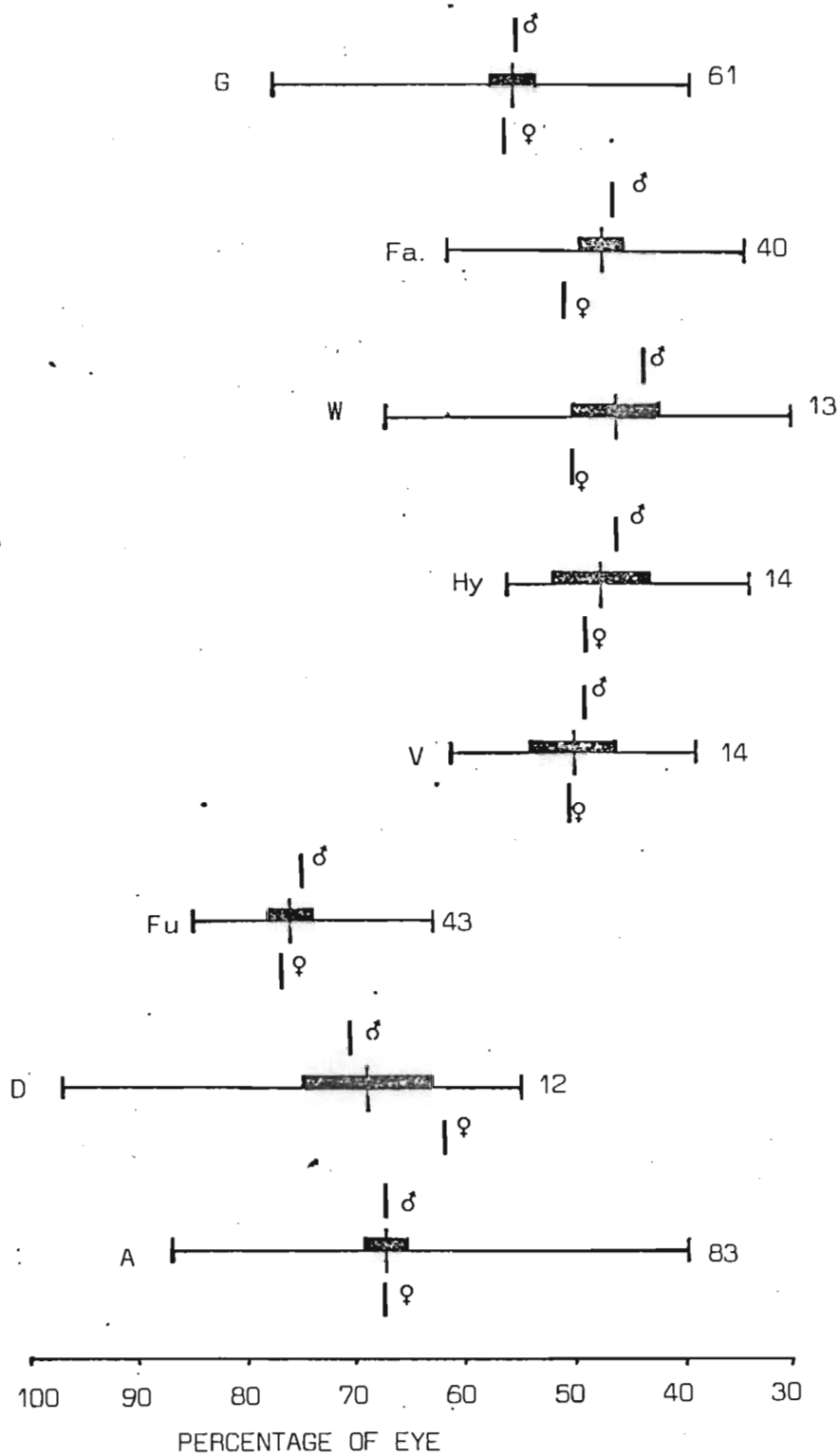


Figure 12. Ratios of tympanum/eye. For explanation see fig. 10.

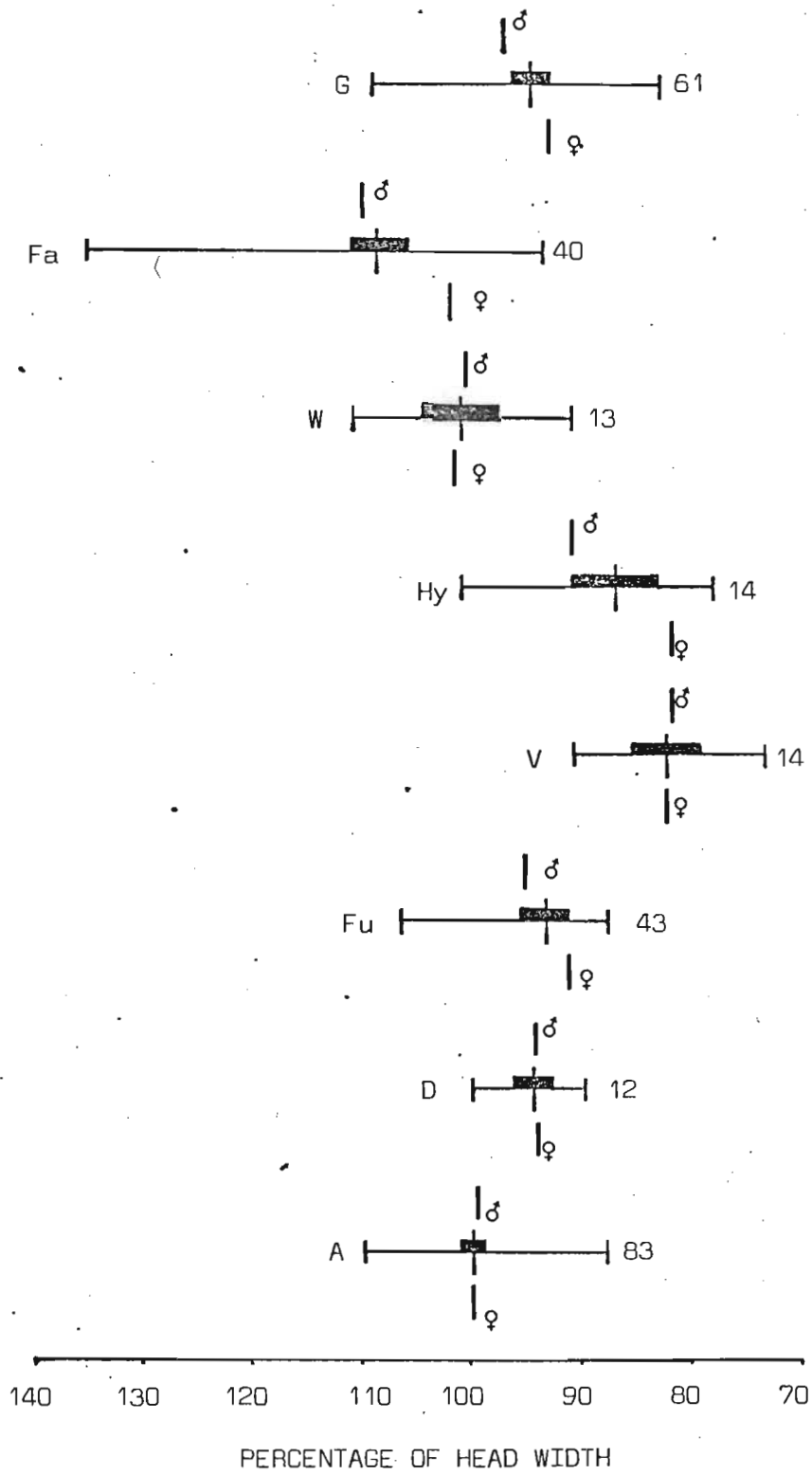


Figure 13. Ratios of head length/head width. For explanation see fig. 10.

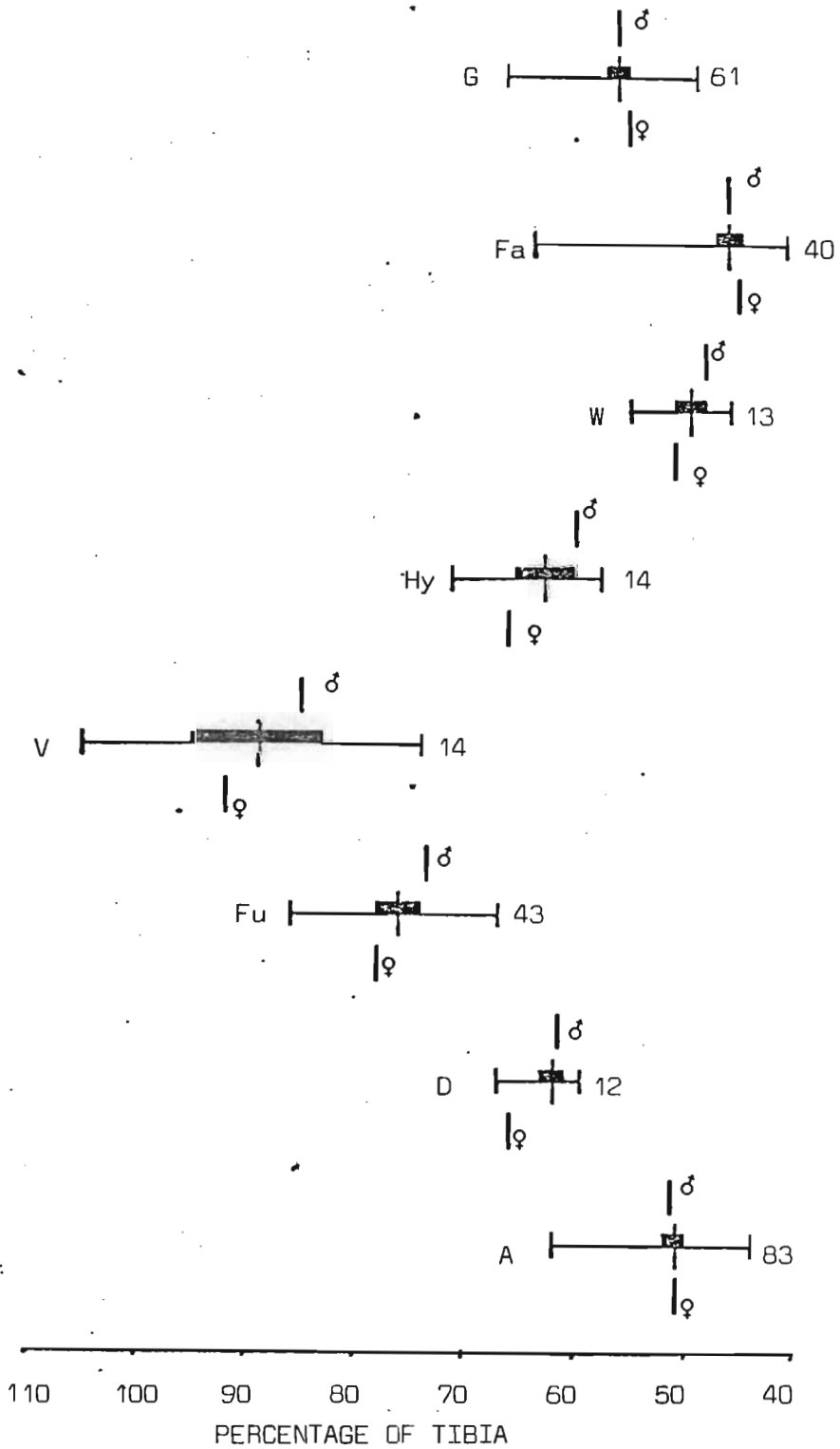


Figure 14. Ratios of head width/tibia. For explanation see fig. 10.



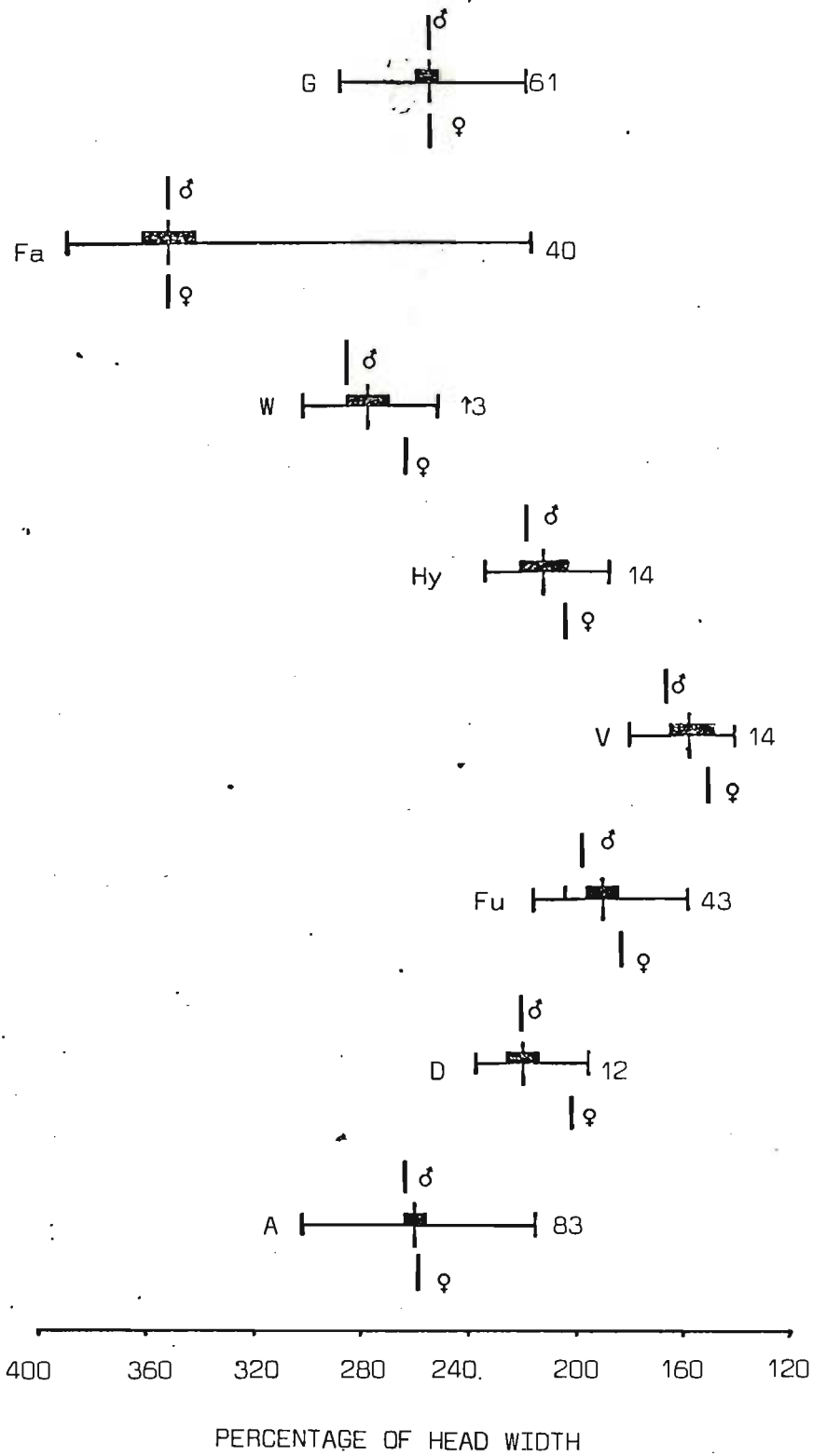


Figure 15. Ratios of foot/head width. For explanation see fig. 10.

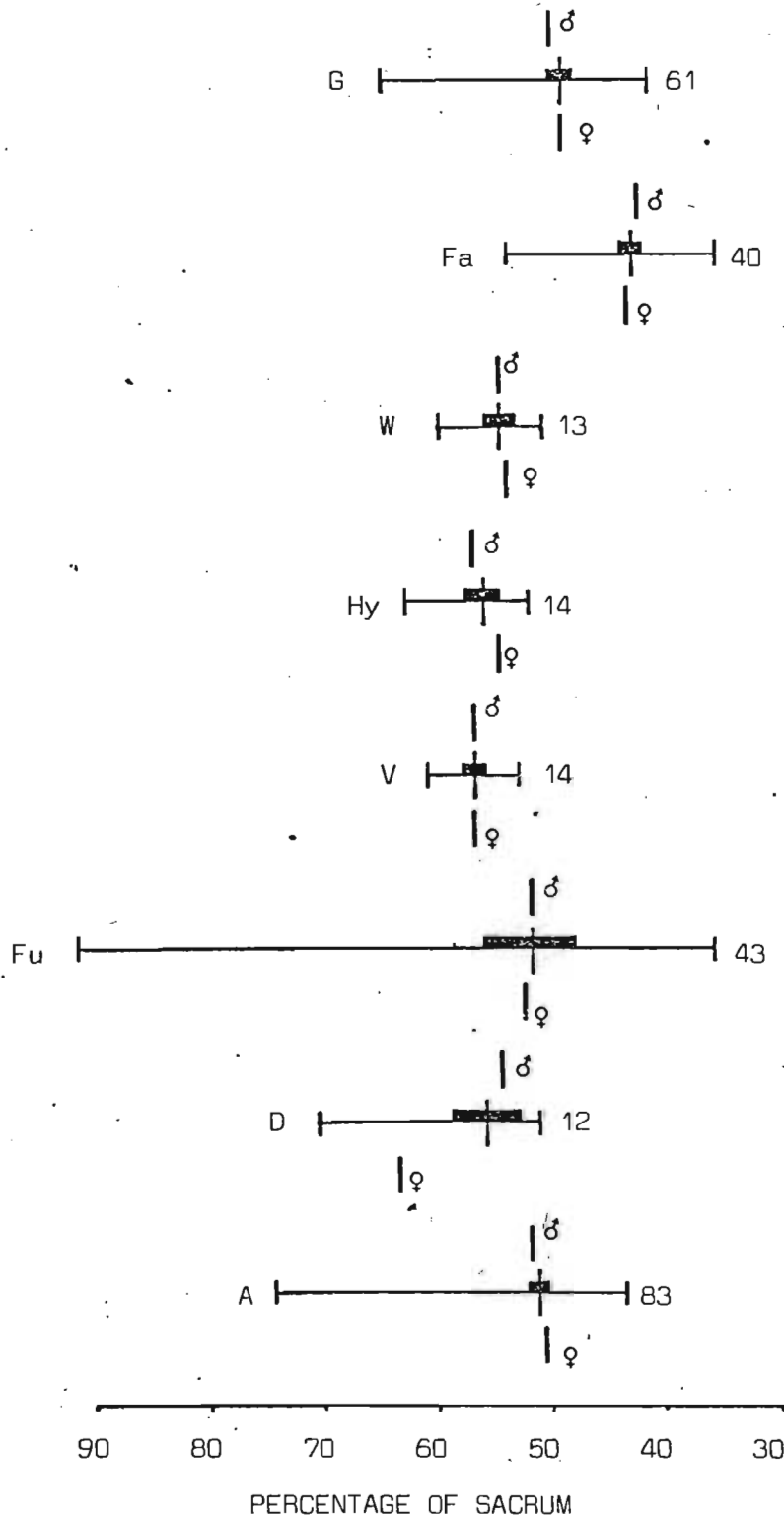


Figure 16. Ratios of ilium length/sacrum. For explanation see fig. 10.

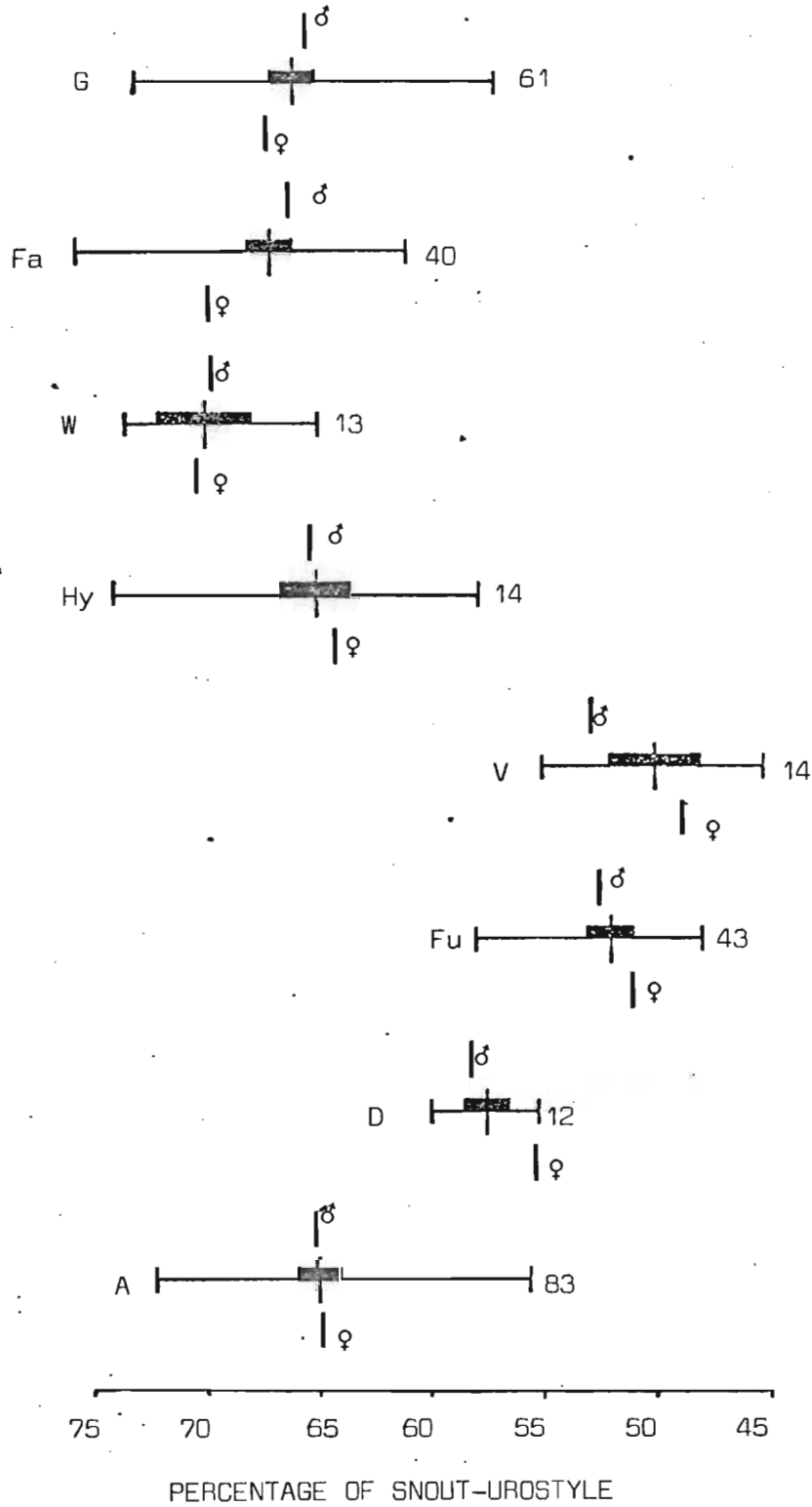


Figure 17. Ratios of tibia/snout-urostyle. For explanation see fig. 10.

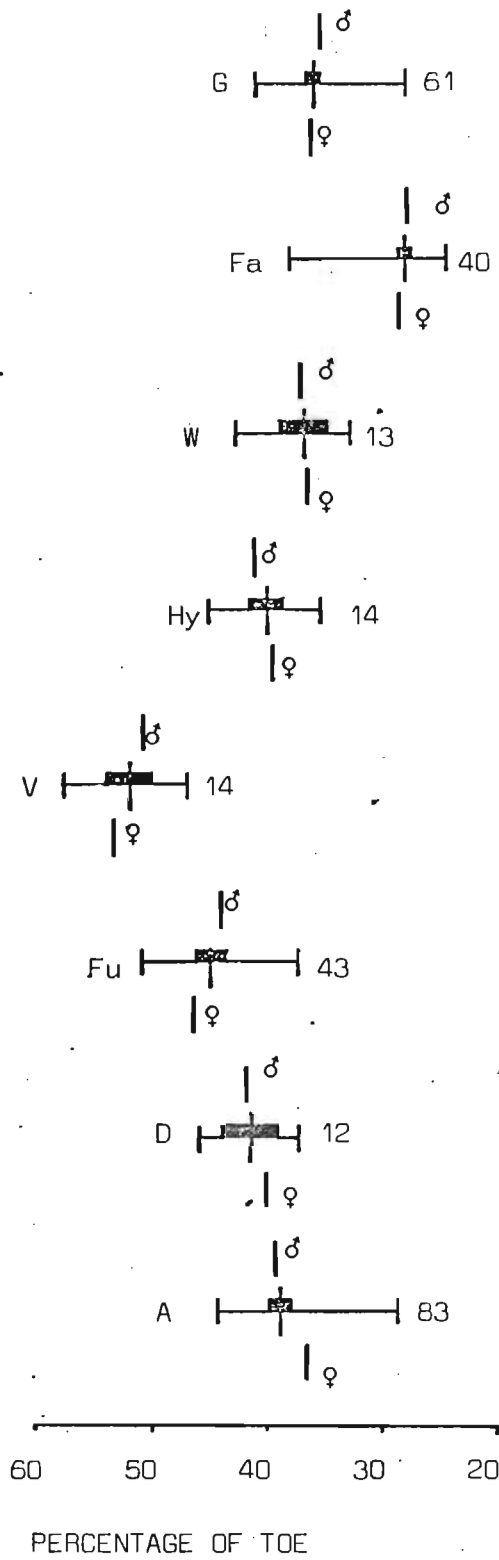


Figure 18. Ratios of ulna/toe. For explanation see fig. 10.



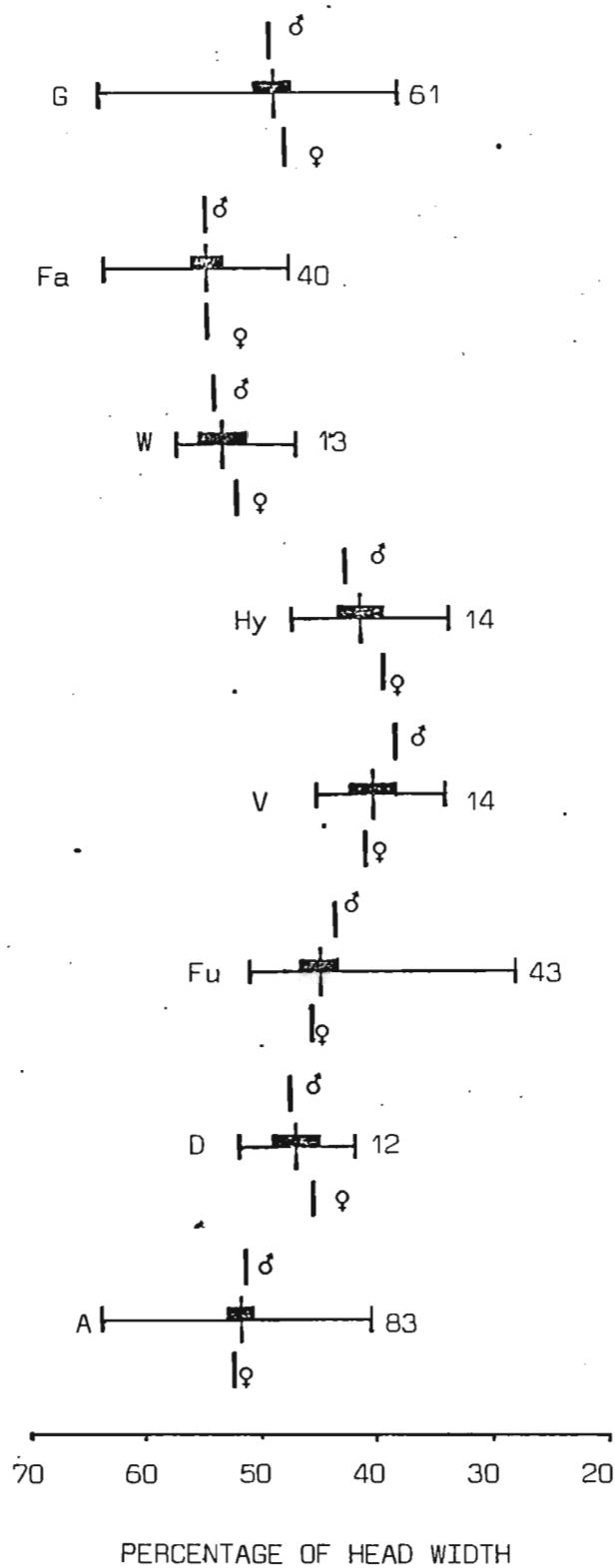


Figure 19. Ratios of head height/head width. For explanation see fig. 10.

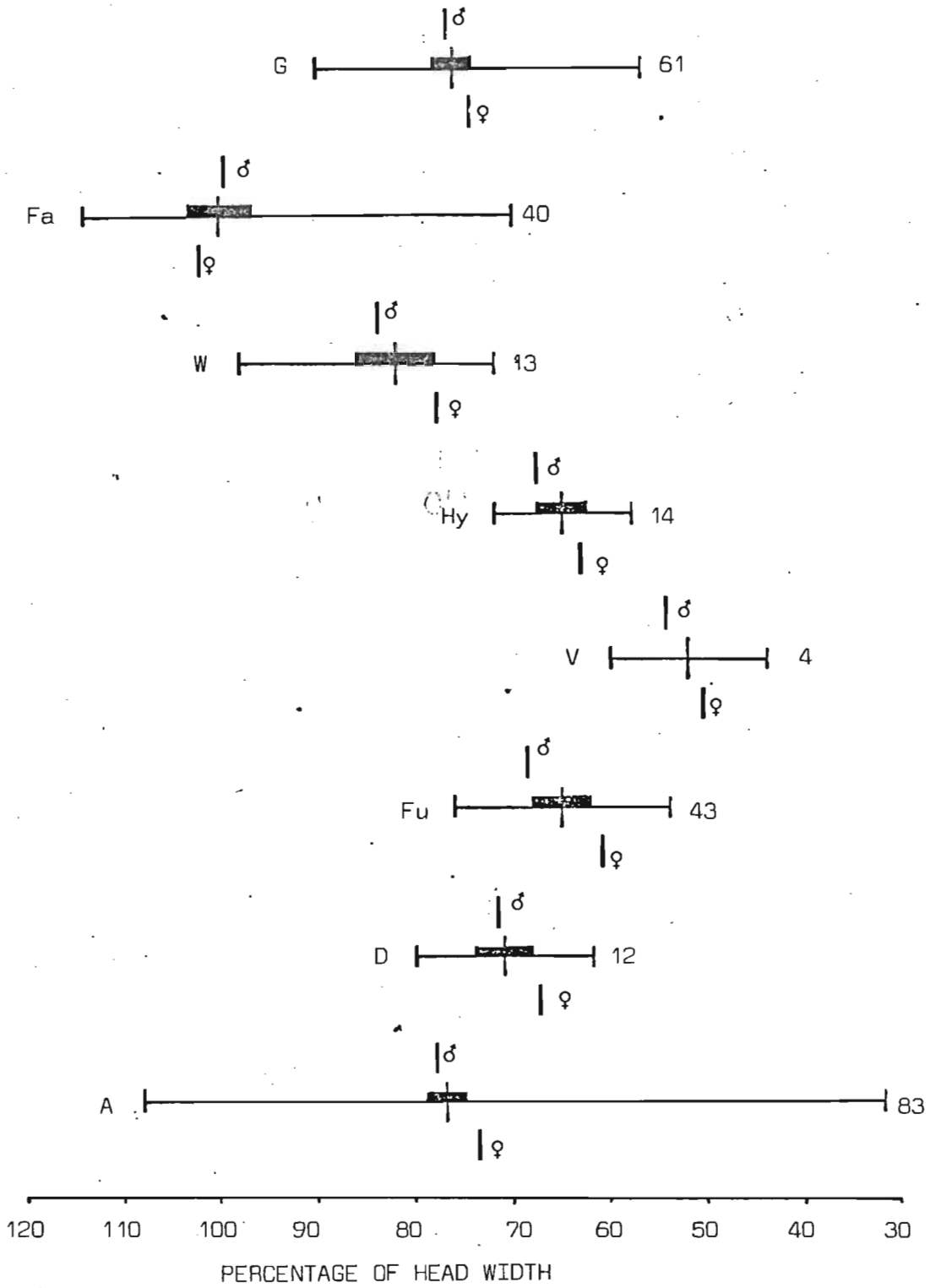


Figure 20. Ratios of hand length/head width. For explanation see fig. 10.

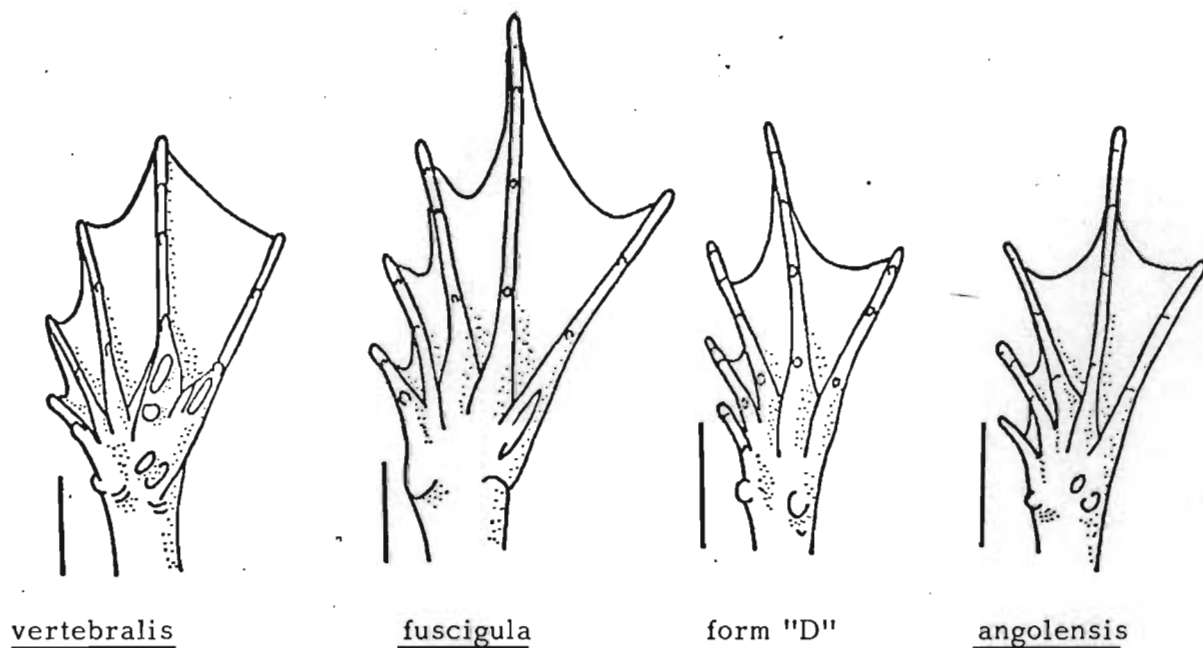


Figure 21. Drawings of the ventral surface of the left foot in representative members of four species: vertebralis, fuscigula, "D" and angolensis. Scale is 10mm.

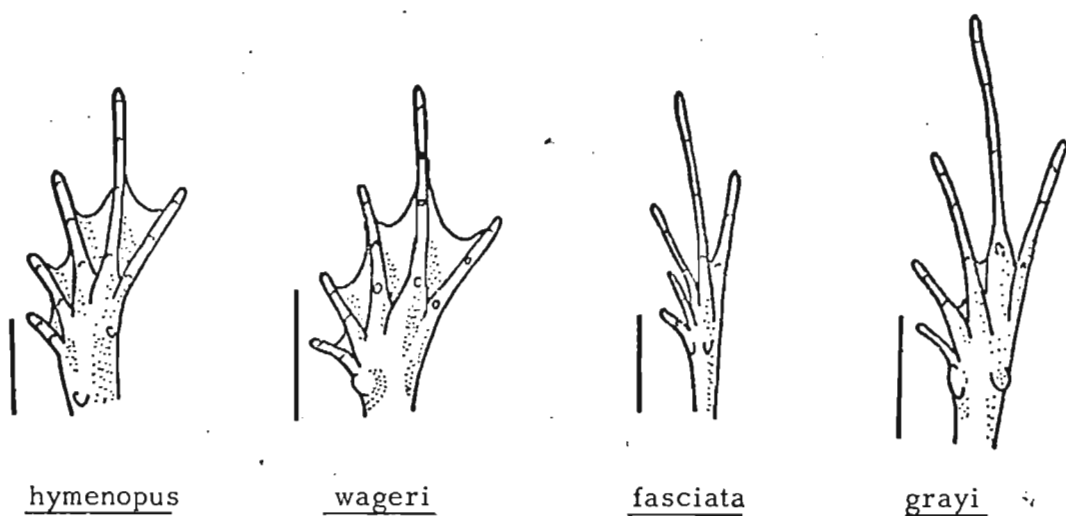


Figure 22. Drawings of the ventral surface of the left foot in hymenopus, wageri, fasciata and grayi. Scale is 10mm.

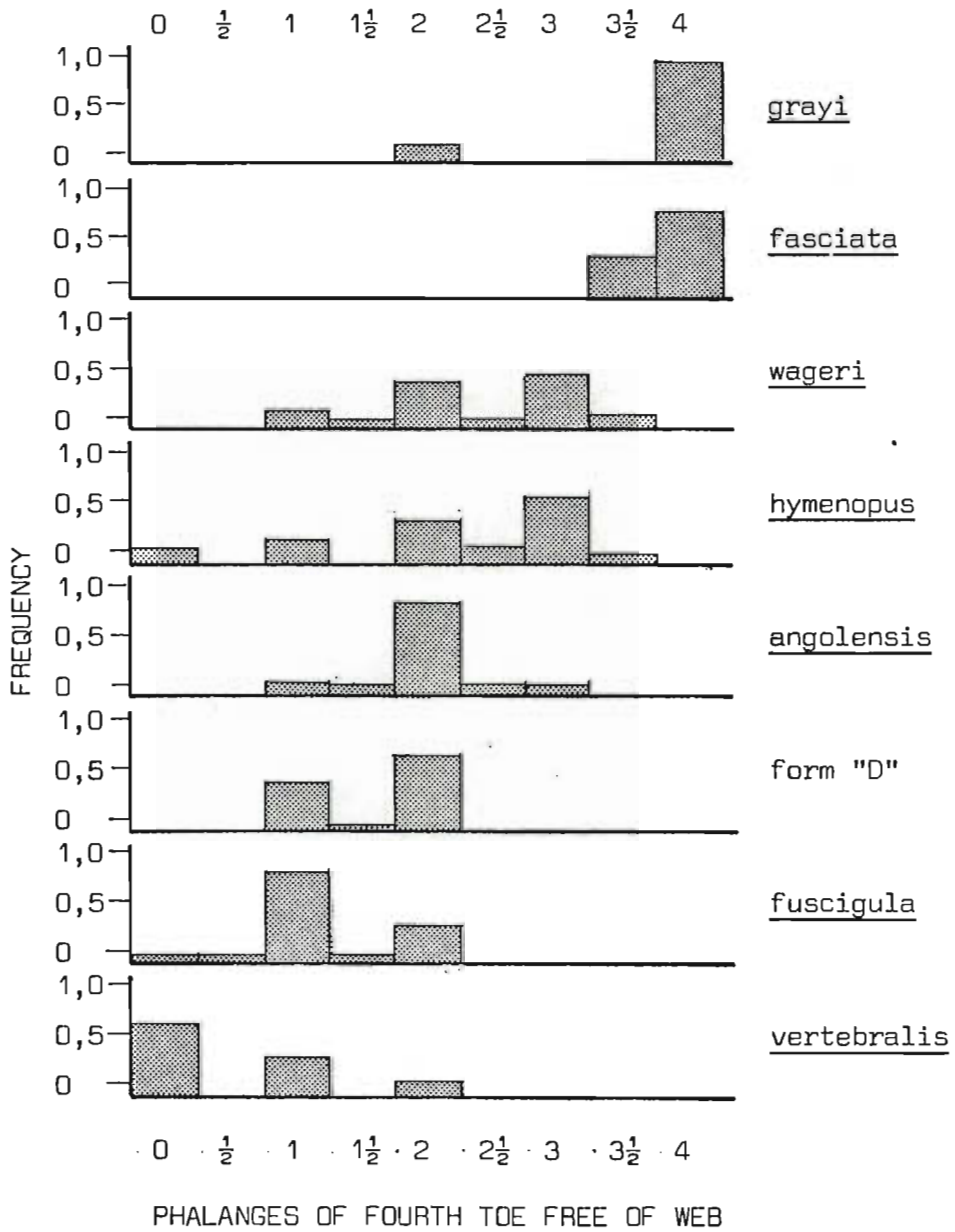


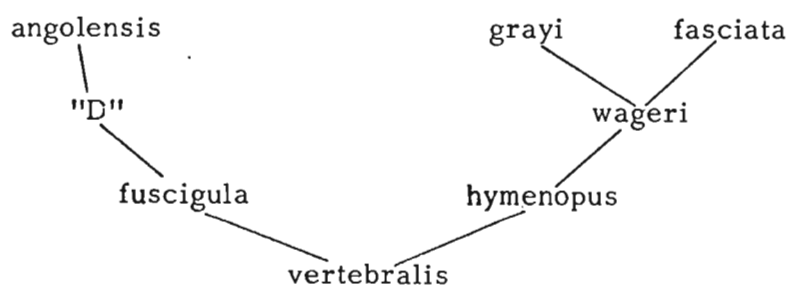
Figure 23. Webbing frequencies as phalanges of fourth toe free of web. Note that the frequencies below 0,1 (10%) have been over-represented for clarity.



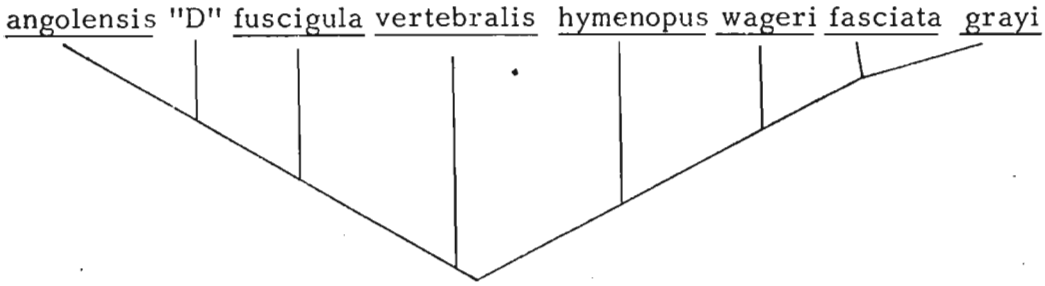
### 3.4 Discussion

The characters and ratios were selected to show differences among the species. No character or group of characters in the R. pipiens complex are considered to be superior for systematic studies, due to the low heritability of linear body measurements (Underhill 1969). Clinal variations in body shape have been shown in R. pipiens to be correlated to mean temperature and the number of frost free days experienced by the animals in the field (Ruibal 1955, 1957). No clinal differences were detected in the species studied.

Two patterns were intuitively selected from the ratios. The first is a stepped series vertebralis - fuscigula - "D" - angolensis which is shown by all the ratios except  $\frac{\text{tympanum}}{\text{head width}}$  and  $\frac{\text{ilia width}}{\text{sacro-urostyle}}$ . The second pattern is a series usually hymenopus - grayi - wageri - fasciata, or hymenopus - wageri - grayi - fasciata. The former pattern shows that vertebralis is more similar to fuscigula, angolensis and "D", while the exception,  $\frac{\text{tympanum}}{\text{head width}}$ , shows vertebralis being similar to grayi, fasciata hymenopus and wageri. The last ratio  $\frac{\text{ilia width}}{\text{sacro-urostyle}}$  shows that all eight species are similar. I have constructed the following phenogram from these two patterns:

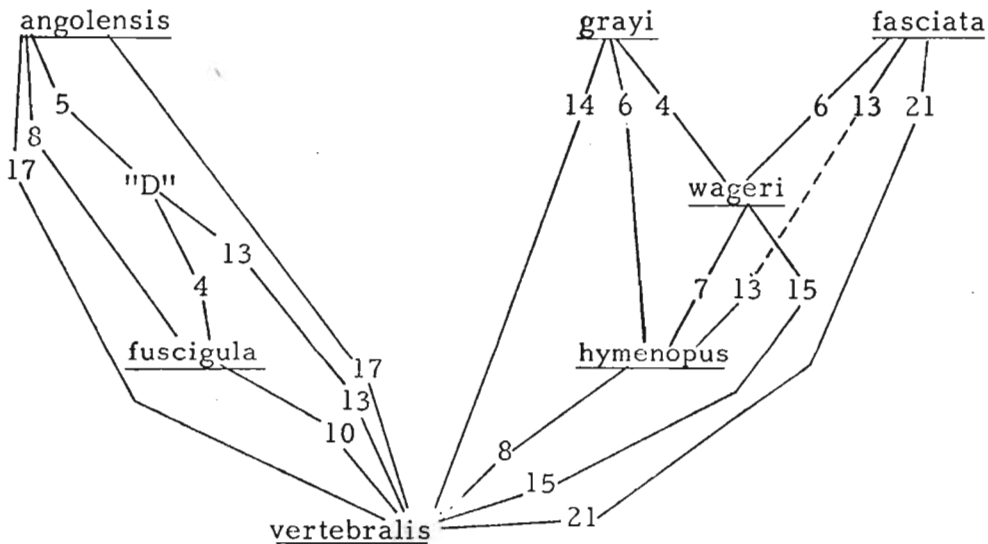


Colless (1970) showed that a phenogram supplies a reasonable phylogenetic hypothesis. I will attempt to show that an optimum phenogram derived from many sources for these species is explicable if the phenogram is regarded as an indication of phylogenetic affinity. I do not mean that any of the species studied are ancestral to any others, but that each species represents a stage in phylogeny reached by the most recent common ancestor. The phenogram above could be drawn like this:



The phenogram above indicates that vertebralis possesses more ancestral states than angolensis, of the characters used to construct the phenogram. The phenogram should be compared to others constructed with different characters, in order to arrive at an optimum design.

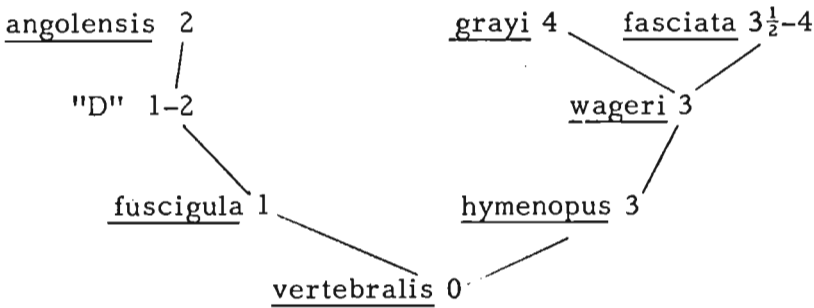
The intuitive relationship described above can be tested by inserting the difference indices ( $D_m$  values) obtained earlier. The  $D_m$  values reflect morphological differences and we could expect more closely related species to have a low  $D_m$  (closer morphological similarity) while more distantly related species have progressively higher  $D_m$  values. Inserting the  $D_m$  values gives the following: ( $D_m$  values are indicated with the decimal ignored.)



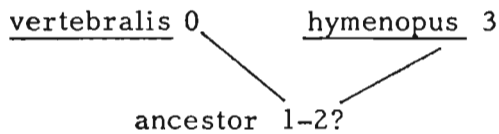
The difference indices fit remarkably well ( $D_m$  increases with distance of relationship). The only exception to this good fit is the similarity of hymenopus and grayi ( $D_m = 0,06$ ) when compared with the proposed closer relationship of hymenopus and wageri ( $D_m = 0,07$ ). This difference is of a low magnitude

as a shift of only  $0,01 D_m$  units would result in a perfect fit. This phenogram will be used to test other data in subsequent chapters.

Inserting the most common webbing values (as phalanges of fourth toe free of webbing), we get:



This shows a transition from vertebralis (0) through fuscigula (1) and "D" (1-2) to angolensis (2). It also shows a transition from hymenopus (3) to wageri (3) to fasciata and grayi (3½-4; 4). This lends further support to the validity of the relationship, but the abrupt change from vertebralis to hymenopus suggests that the two should be separated proportionately more than any other pair. The problem would be solved if we place hymenopus and vertebralis at the bases of two separate lines, joined by an ancestor possessing presumably intermediate characters:



The eight species cover the full range of webbing possibilities, from fully webbed to completely web free. As a group they are thus highly diversified. The ecological implications and correlations will be discussed later. It must be emphasized that this is only one way of looking at past evolutionary events in terms of existing populations. Morphological specializations may completely mislead the observer, but if we test as many lines of evidence as possible (ecological, behavioural, distributional among others) it should be possible to arrive at a reasonable hypothesis concerning the relationships of the group.

#### 4 MATING CALL

An appraisal of the nomenclature and function of various anuran vocalizations has been presented by Passmore (1977). The calls described in this report are associated with breeding, and are called mating vocalizations.

##### 4.1 Recording and analysis of calls

Mating calls and territorial calls were recorded in the field using Uher 4000 series tape recorders, or a Uher CR 210 recorder. Three species (vertebralis, hymenopus and wageri) were recorded in an aquarium in the laboratory. A directional "shot gun" microphone (AKG 90) was used for most recordings as it permitted the isolation of vocalizations from one individual in a chorus. Water and air temperatures were taken at most sites. All the calls were analysed on a Kay 7029A Sona Graph. Terminology is explained in the appendix.

##### 4.2 Results

The sites at which calls were recorded, or from which specimens were collected for later recording, are shown in figure 24. The mating calls of these frogs can be divided into three types. The first type is biphasic, with an initial slowly pulsed "clicking" phase, followed after a pause by a rapidly pulsed phase - a pattern shown by vertebralis, fuscigula, "D" and angolensis. The second call type consists of a series of single or double pulses which may be strung together into various lengths of pulse-trains and is shown by hymenopus and wageri. The third call type consists of a single pulse, uttered at intervals, which is characteristic of grayi and fasciata.

The call of vertebralis (fig.25). Length of initial phase 3-11s (mean 7,5), pulse length 3-5ms, pulse rate 6,2-8,4/s (mean 7,4). Harmonics between 0,1 and 1,1 kHz, usually 0,1 and 0,4 (or 0,2 and 0,5 kHz) are dominant. Mean dominant harmonic 0,5 kHz. The interval between the first and second phases is 0,29-0,54s (mean 0,37), but may be as long as 3s when the frog changes emphasis from first to second phase calls. The second phase has a duration of 0,03-0,57s (mean 0,29), a pulse rate of 190-225/s except in calls over 0,45s when the pulse rate drops to 145/s after an initial burst at about 210/s. Mean pulse rate for the second phase is 211/s. The second phase is vocalized between 0,08-0,8 kHz with harmonics at 0,1 (or 0,2) and 0,4 (or 0,5) kHz. Mean dominant harmonic 0,2 kHz.



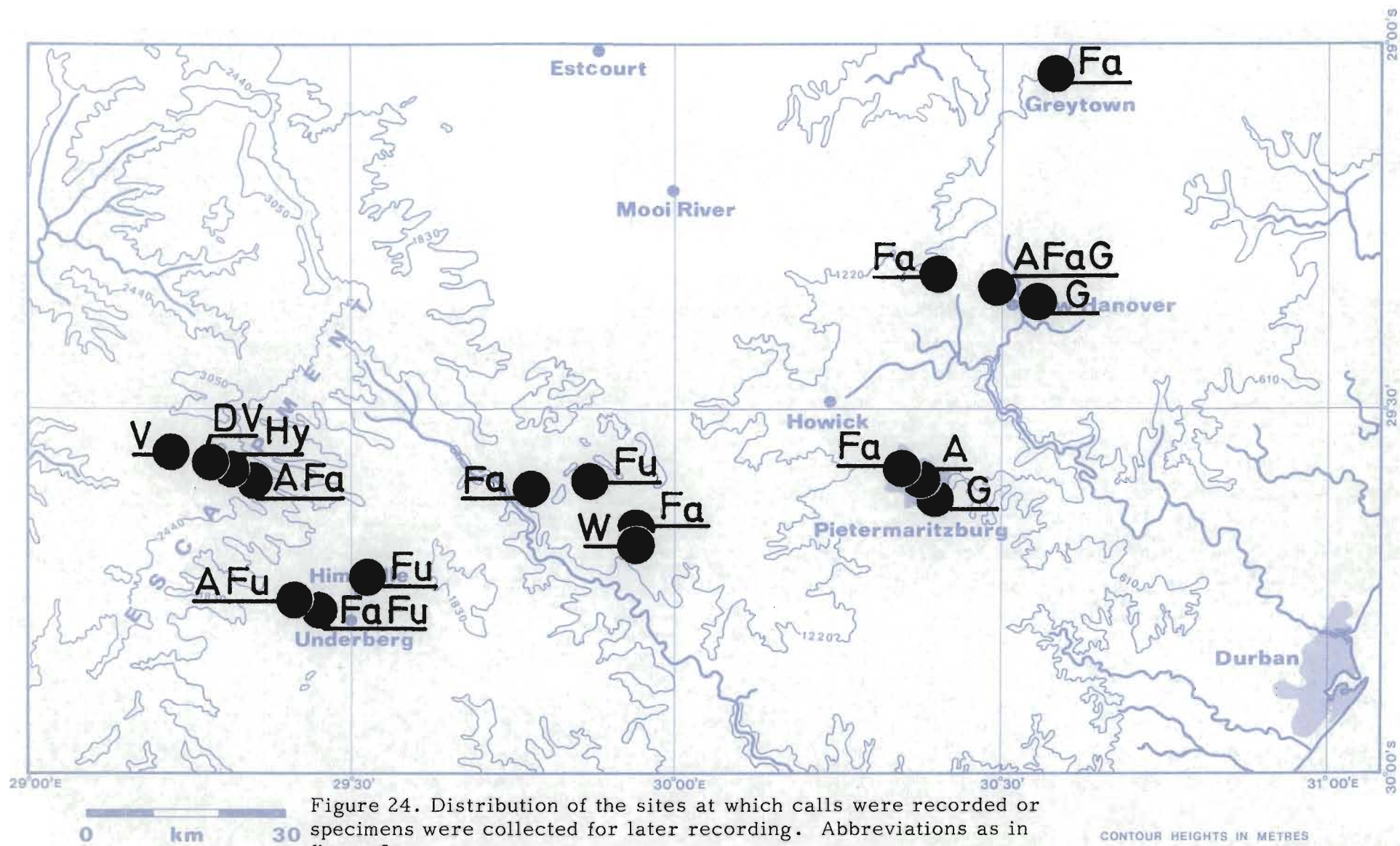


Figure 24. Distribution of the sites at which calls were recorded or specimens were collected for later recording. Abbreviations as in figure 3.

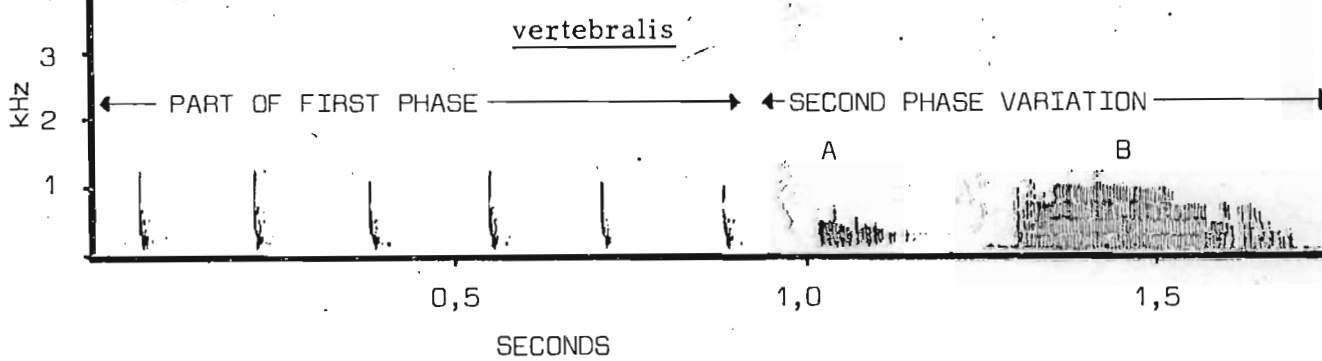
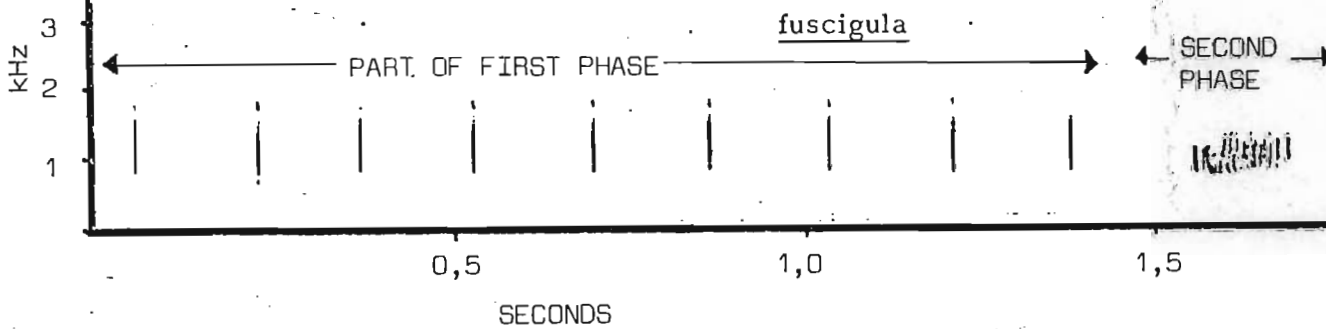
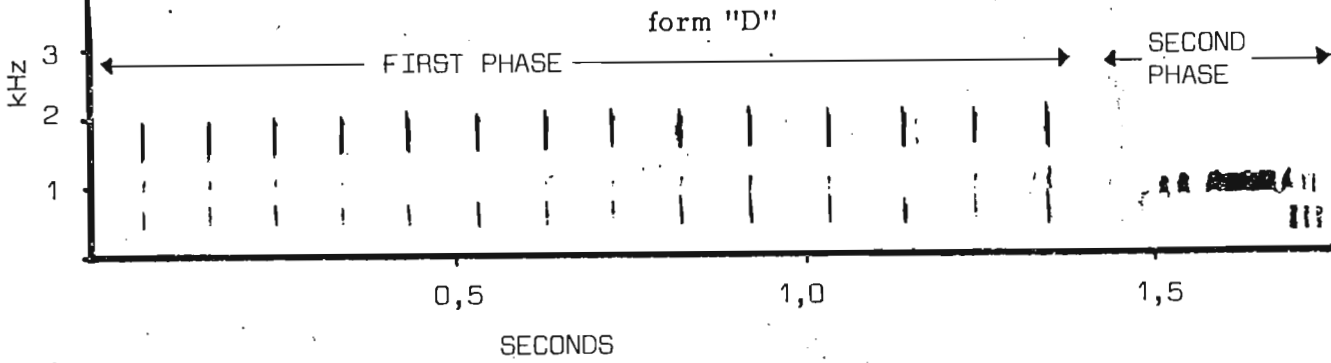
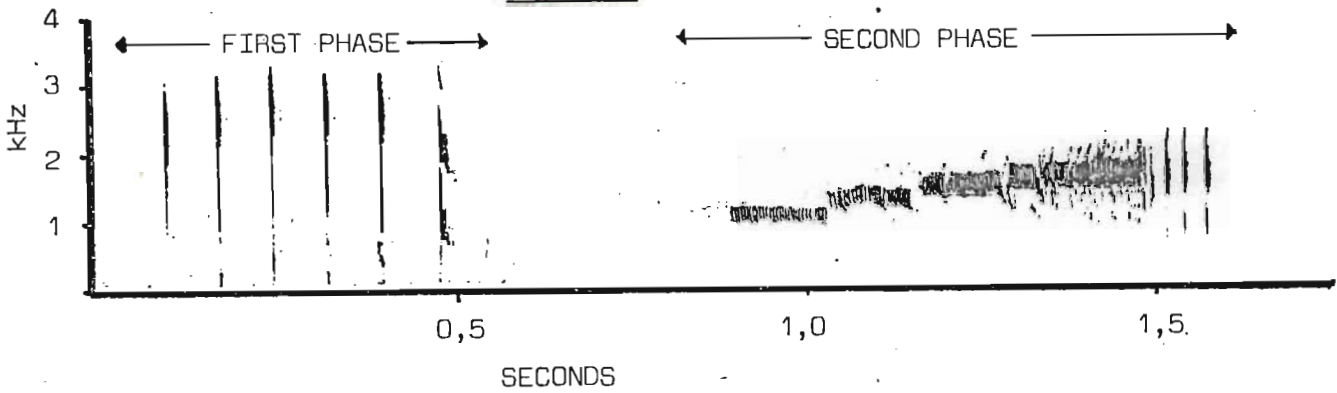
angolensis

Figure 25. Sonagrams of representative calls or parts of calls. Note the FM tendency of the second phase of the angolensis call, and the variation in vertebralis second phases. The short version is closely associated with the first phase, while the longer version (B) is sometimes uttered alone.

The call of fuscigula (fig. 25). The length of the initial phase varies from 8 to 50 seconds (mean 28). Length of each pulse 4–8ms. Pulse rate of initial phase 3,7–9,5/s (mean 6,2). Frequency of the initial phase 0,3–1,6 kHz (mean 1,0). The interval between the first and second phase varies from 0,7–2,0s. The second phase has a duration of 37–302ms (mean 110), a pulse rate of 110–265/s (mean 174) and is vocalized at 0,3–1,6 kHz (mean 1,0).

The call of form "D" (fig. 25). Duration of initial phase 0,8–1,3s (mean 0,99). The individual pulses are 7,5ms long and are uttered at a rate of 9–19/s (mean 12). The dominant frequency varies between 1,7–1,9 kHz, with a harmonic between 0,6–0,8 kHz. The interval between the initial and second phases is 75–120ms. The second phase has a duration of 143–603ms (mean 389), a pulse rate of 42–46/s (mean 44). Harmonics are at 0,4 and between 1,0–1,2 kHz. The second phase shows frequency modulation (FM) at the end of the call.

The call of angolensis (fig. 25). Duration of initial phase 159–868ms (mean 353), pulse rate 8,9–16,6/s (mean 12,4), frequency usually between 1,5–2,7 kHz, occasionally lower. Interval between the initial and second phase 0,2–3,0s. The second phase is 0,2–1,3s long (mean 0,54), with a pulse rate of 87–162/s (mean 112). The pulse rate is not always easy to calculate, especially in the sonagrams showing an FM second phase. Two harmonics at 0,8 and 1,6 kHz are usually present, although the energy in the call varies between 0,7–2,7 kHz in some individual's calls.

The call of hymenopus (fig. 26). The call consists of single or double pulses which may be uttered discretely, or may be strung together to form pulse trains of various durations. The mating call often becomes louder with longer pulse trains when it presumably takes on a territorial function. As the two functions are apparently performed by either end of a continuum (in terms of call structure) it is not possible to clearly delineate where the mating call ends and the territorial function takes over. Passmore (1976, 1977) discusses the recognition and function of various frog calls. Call duration varies from 7,5ms (single pulse) to 53ms (pulse train). Pulse rate 133–266 (mean 209)/s. Dominant harmonic at 0,9–1,2 kHz (mean 1,0).

The call of wageri (fig. 26). The mating call consists of units which each contain one to six pulses, commonly two. These units are in turn strung together to form pulse trains of various lengths. As in

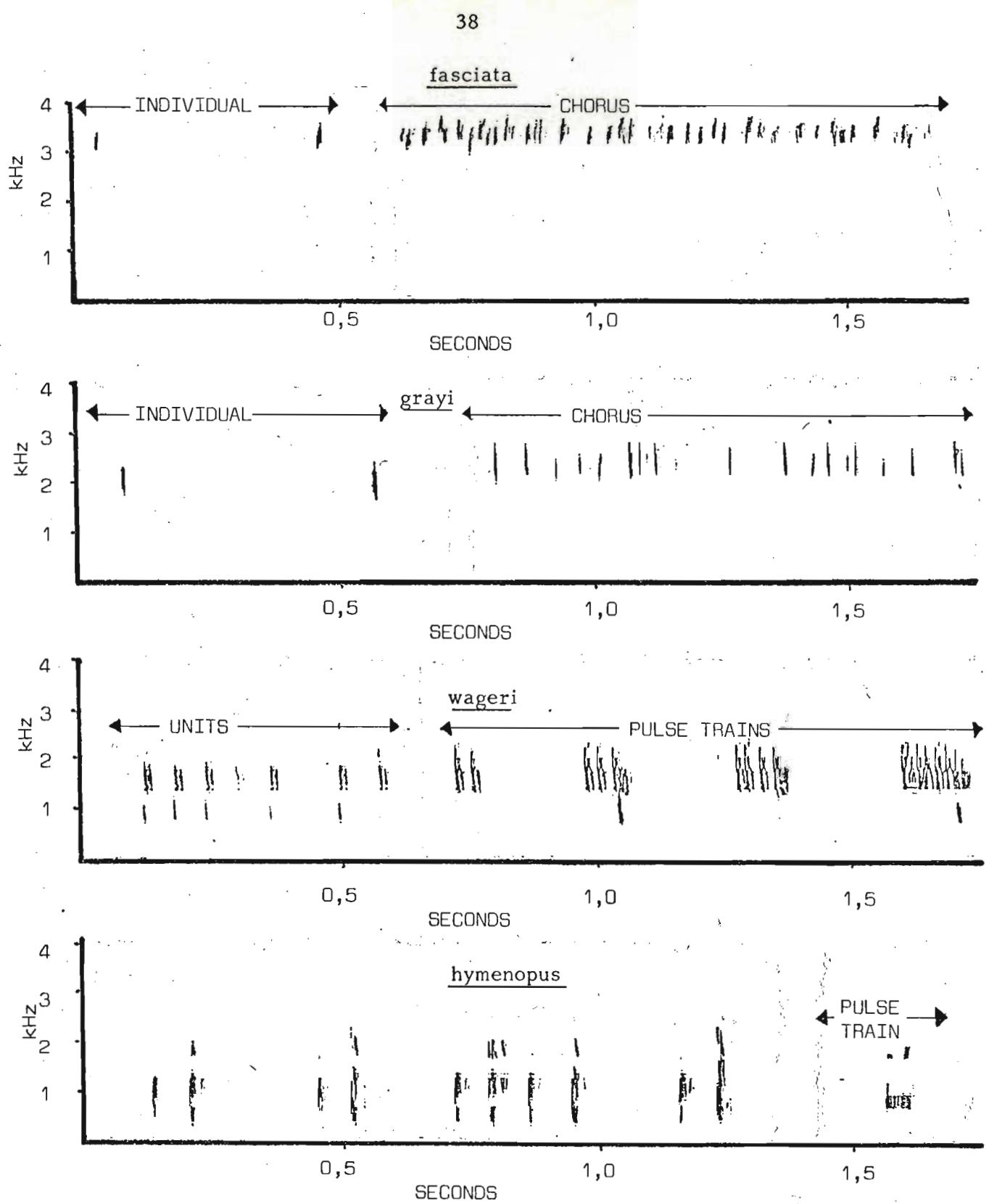


Figure 26. Sonagrams of the mating calls of fasciata, grayi, wageri and hymenopus.



hymenopus, there is a shift from units to longer pulse trains with an increase in amplitude and frequency as the call takes on a territorial function. Duration of one unit 11,3–37,7ms (mean 18,9). Duration of pulse train 22,6–1298,1ms (mean 267,7). Pulse rate 158,9–219,0/s (mean 193,9). Dominant harmonic 1,6–2,3 kHz, normally two or three other harmonics present. A call with harmonics at 1,6; 1,8; 1,9 kHz with 1,8 kHz dominant, is common. Intercall interval 52–377ms.

The call of grayi (fig. 26). The call consists of a single or double pulse with a duration of 3,8–13,6ms (mean 6,3) at a frequency of 1,6–3,1 kHz (mean 2,3). The territorial call in grayi is of a longer duration (22,6–30,2ms) and at a lower pitch (1,3 kHz). It may be this call which Poynton (1964a) regarded as a character indicating taxonomic difference between upland and lowland populations of grayi, as it is very easy to distinguish between the two calls in the field. Although one or other may be more common at certain times (especially if the observer only listens briefly) both can be heard at ponds where breeding aggregations are present.

The call of fasciata (fig. 26). A pulse duration of 7,5–22,6ms (mean 9,3) at a frequency of 3,1–3,8 kHz (mean 3,3) distinguishes the call of this species from that of grayi. Harmonics are sometimes discernable at 3,2 and 3,4 kHz.

The mean values for frequency, pulse rate and duration are compared in figure 27.

	Fa	G	W	Hy	V	Fu	D	A
Frequency (dominant harmonic)	3,3	2,3	1,8	1,0	0,5 0,2	1,0 1,0	1,8 1,1	2,0 0,8
Pulse rate (per s)	–	–	194	209	7,4 211	6 174	12 44	12 112
Duration (s)	0,009	0,006	0,267	0,236	7,50 0,29	28,00 0,11	0,99 0,39	0,35 0,54
n	382	166	46	24	12	83	14	91

Figure 27. Table of mean values of comparative mating call parameters. Abbreviations as in fig. 9. Where two values are given in one block, the top value refers to the first phase, and the lower value to the second phase.

### 4.3 Adaptations of vocalizations

Reviews of the functions and adaptations of anuran vocalizations have been presented by Bogert (1960) and Blair (1963). The primary function of the vocalizations of anurans is to attract conspecific females. Associated with this is the possession of a species-specific mating call. The ways in which mating calls are structurally related could provide evidence of their evolutionary history.

The call of hymenopus is a simple "on-off" amplitude modulated vocalization (AM) which operates at a fixed frequency. This appears to be a simple and effective form of communication, as similar calls are known for many other anurans. However, the call is very quiet, and in the absence of any background noise is not audible (to me) at distances in excess of about five metres. This contrasts with the calls of genera like Bufo and Tomopterna which can be heard at hundreds of metres. A possible explanation may be found by examining the sound environment in which this species lives. The mountain streams where this form occurs create background noise in the form of splashing water from rapids and waterfalls in the frequency range from 0,4-1,0 kHz. This overlaps partially with the dominant frequency of the call of hymenopus (0,9-1,2 kHz). This species may have a soft call to avoid competition with the background noise i.e. to avoid wasting energy making "waterfall-like noises" which would only be lost against the background. Males and females have only been found near running water during the breeding season. They are not far ranging like the savanna Bufo spp with loud calls. It appears that hymenopus relies on close-range attraction utilizing a pulsatile vocalization as it is only in the ability to modulate amplitude regularly that this species' call differs from the random nature of the background noise. It is remarkable that this species should live in such apparently unsuitable sound environments. In terms of distribution and apparent numbers this species is one of the less successful of the group.

The next species, wageri, calls very quietly like hymenopus, and also lives in similar situations where rushing water is a constant feature of the sound environment. However, wageri calls at a frequency a little above that of the environment. Males and females tend to be found underwater in shallow pools when they are not on the surface breeding. Wager (1965) records that males sometimes call while submerged. Perhaps males which call underwater avoid competition from air-borne sounds.

They do call from conventional sites at the water's edge as well. Like hymenopus, wageri is presently found in very restricted areas, and does not seem capable of expanding its range.

The simplest call structures are found in the calls of grayi and fasciata. Both calls consist of a single or double pulse only a fraction of a second long, uttered at relatively high frequencies. The call of fasciata is louder than that of grayi, and is heard at greater distances. An obvious advantage of an ultra short call is that a large number of males can call together at one site without overlapping. On the sonagram (fig. 26) it can be seen that over 25 calls per second are common. Both these species are successful in terms of distribution and numbers. Although grayi is often found near running water, it calls at a frequency high enough to avoid any overlap with environmental noise.

Another species with a quiet call and restricted distribution is vertebralis. The call of this species is biphasic; both phases are amplitude modulated only. The second phase has the lowest mean frequency of all the species in this group (0,2 kHz). The functions and advantages of a biphasic call over a single phase call are explicable in terms of information carried and behavioural adaptations. A longer, more complex call contains more specific information about the identity of the caller. Because species-specificity is encoded in the female auditory discriminatory ability as well as in the vocalization of the male (Mudry et al 1977), biphasic calls may reduce the chances of mismating more than a single phase call. Many hybrid populations are known where the mating call was obviously not an efficient pre-mating isolating mechanism (Passmore 1972). In Passmore's study, the species of toads involved all possessed single phase calls. Bogert (1960) lists other hybrid populations, all of which derive from parental species with single phase calls. The behavioural advantages of biphasic calls will be discussed below (see paragraph on angolensis).

The vocalization of fuscigula is the longest in the group, the first phase has a mean duration of 28s. This loud pulsed call is audible for many hundreds of metres. Other males utter the second phase while one is vocalizing the first phase. Remarkably, both phases are at the same frequency (1,0 kHz) which indicates that the difference in pulse rate between the phases (6,2/s; 174/s) is adequate to distinguish them. The short (110ms) duration of the second phase means that it can be uttered between two consecutive pulses of the first phase, so that minimal temporal overlap

occurs. The loud first phase is probably an adaptation to the flat marshy areas where this frog occurs, as it may serve to attract females and males from relatively large distances to localized breeding aggregations. My observations indicate that the first phase is heard where there are large breeding aggregations, while the second phase is heard in situations where there are only a few individuals. Hewitt (1937) notes that the loud drumming call is heard during the breeding season in the Cape. In Natal they may breed throughout the year, but peaks of activity are found in spring and early summer. Fuscigula is a successful species and possesses an effective first phase for attracting conspecifics. A study of male-male interactions, use of the two phases of call, and success at breeding of males using the different calls would be a most rewarding task.

"D" has a shorter first phase than fuscigula (0,99s) but a relatively more important second phase. The second phase of "D" is 39% of the length of the first phase, while in fuscigula the second phase is only 0,39% of the first phase! As stated earlier, the function of the two phases needs to be checked experimentally, which is beyond the scope of the present study. The second phase is frequency modulated. Some of the possible advantages of FM calls will be discussed below.

The short first phase of the angolensis call consists of only five or six pulses. The second phase is more elaborate than in the calls of other species with biphasic calls. It is also longer than the first phase. Although no experimental evidence is available, from field observations it appears that the first phase uttered by one male inhibits the use of the same phase by other males nearby. These other males utter the second phase which may be important for close-range attraction. I believe that the FM component enables a female to "tune in" to a changing note, to avoid confusion with other nearby males which are unlikely to be calling at the same frequency or with the same rate of frequency change. In a small chorus of six or seven males it is not uncommon to hear a particular male take the lead with the first phase, while the other males fill in with the second phase. An alternation of phases results as a second male will take over the first phase call while the 'leader' is uttering the second phase. The overall sound picture is of first phases uttered continuously by different males, rarely overlapping, while the rest of the males in the chorus utter the second phase simultaneously but asynchronously. This pattern may be heard in any pond where angolensis is calling, and results in a sophisticated

time and frequency sharing of the available sound environment.

Coding the mating call with spectral energy (FM) in this way is only useful at short distances, because high frequencies attenuate faster than lower frequencies. FM coding is therefore mostly used in animals which gather for breeding (Konishi 1970). This supports the view that the second phase in angolensis is a short range attractant.

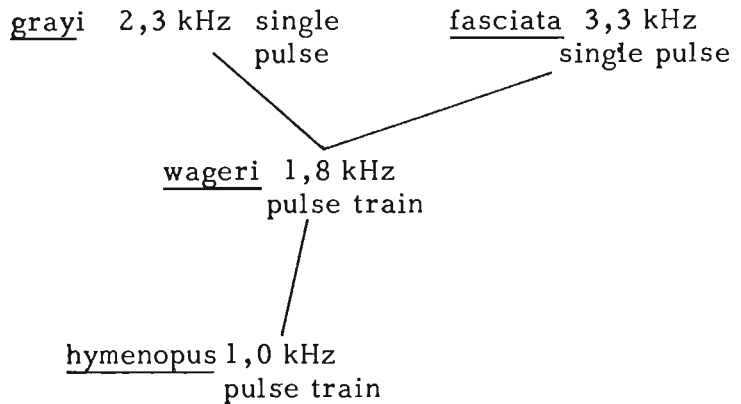
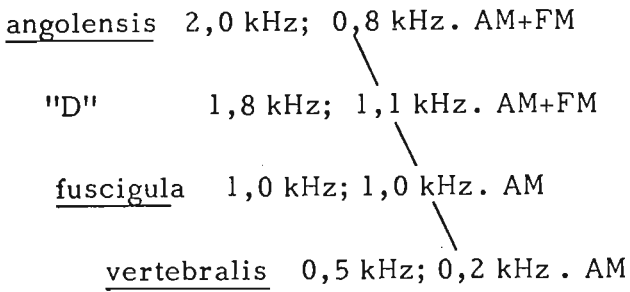
North American R. pipiens has a triphasic call (A,B,C). Males respond with call C after hearing A. C calls inhibit calling in other males (Pace 1974). Pace presented experimental evidence of the functions of these calls, from which she concluded that A is a long range attractant (A is also the longest phase) while B is a close range direction finder. C appears to be an aggression call, probably with a spacing function between males (Pace 1974).

#### 4.4 Discussion

Anuran vocalizations have been used as indicators of the presence of cryptic species (Brown and Brown 1972, Littlejohn and Oldham 1968) because of their species-specificity. This specificity has been shown to depend upon frequency (Straughan 1973) and pulse repetition rate (Straughan 1975). Straughan showed how Hylid frogs optimize detection of the call by matching the frequency of best hearing with the dominant frequency of the call, while pulse repetition rates are used to discriminate between species. Even male release calls, which are uttered when a male is clasped by another male, are species specific (Schmidt 1976).

Analysis of the mating calls of the eight species studied indicates that they all utilize vocalization as a pre-mating isolating mechanism. The calls can be used to divide the species into two major groups; those with biphasic calls (vertebralis, "D", fuscigula and angolensis) and those with simple AM calls which may be formed into pulse trains (wageri, fasciata, grayi and hymenopus). This supports the earlier grouping of the species on morphological grounds (section 3.4). Martin (1972) and Tandy (1976) show that call structure may be used to discern phylogenetic relationships. When overlaid on the phenogram used earlier, it can be seen that a stepped pattern emerges, where the emphasis in the vertebralis-fuscigula-"D"-angolensis group shifts from AM to FM endings, with an increase in the frequency of the first phase. The second group shows a reduction of pulse trains to single pulses and an increase in dominant frequency:





Japanese ranas show a similar pattern, where the short mating call of R. nigomaculata may be derived from the long mating call of R. brevipoda (Kuramoto 1977). In contrast to the above pattern which suggests that the line leading to the evolution of angolensis gained an FM component, Bufo are believed to have evolved from a leptodactylid stock, losing the FM pattern in favour of a stabilized AM pattern of calling (Martin 1972). From the above pattern it might be suggested that high frequency calls represent the ancestral state which has given rise to the low frequency calls. However, territorial calls tend to have higher dominant harmonics than mating calls in this group. It is possible that the ancestral state of the character was low frequency, which permitted the new species call to develop out of a pre-existing territorial call, thus simultaneously repelling individuals of the ancestral species (if there was any contact) while attracting individuals with the new genetic makeup. Evidence from mating and aggression call structure indicates that the mating call of R. utricularia derived from the aggression call of R. pipiens (Pace 1974). Speculations like this may indicate the process involved in the evolution of a new anuran species and the reinforcing role played by vocalization.

The biphasic call of the vertebralis-fuscigula-"D"-angolensis group may have evolved at a time when the ancestral form was very abundant and might have utilised a separate male aggression call for spacing males, besides having a female attracting call. Under conditions of crowding at the breeding areas it would have been advantageous for both calls to be incorporated into one unit, so that by a simple behavioural adaptation male-male repulsion and male-female attraction was combined into a biphasic call. Animals possessing this biphasic call would have had an advantage over individuals who uttered both calls separately, and were presumably selected for their efficient vocalization. On the other hand the biphasic call may have evolved purely as a means of increasing the complexity of the call to improve the species-specificity in an environment where many other anuran species were already established.

Discrimination tests to determine the functions of the two phases are required. The variables in such a test where animals are exposed to calls emitted from a loudspeaker in an attempt to observe behavioural modifications in the listener, are daunting. Temperature, humidity, reproductive state of experimental animals, reproductive state of animal whose calls are used, sound pressure levels of the call, time of day and season and previous experience of the experimental animals are important variables to be considered.

The parameters of male mating calls may be selected for by abiotic and biotic pressures. Biotic pressures would include the existing calls in the area while the environment may be an important selection pressure. Morton (1975) studied avian sound environments and showed that the optimum frequencies for ground dwelling birds in forest to achieve maximum broadcast area lie between 1000 and 2500 Hz. The only frog in the group confined to forest, wageri, which calls from ground level, has a mean emphasized frequency of 1800 Hz, which is practically in the middle of the optimum sound window in the forest environment. Where there is an open space for sound to travel without obstruction, higher frequencies and wider frequency ranges are more favourable for transmission. Birds singing on the wing or in tree tops reduce the attenuation of high frequencies. Such birds usually sing between 4-8 kHz (Konishi 1970). This study suggests that the reason why fasciata calls from vegetation above ground level is to reduce the attenuation of the high frequency of the mating call (fasciata calls at 3,3 kHz - the highest in this group).

Species in this group with quiet calls are restricted to localized patches of suitable habitat, or to areas like the Lesotho plateau which are remarkably uniform.

The structural relationships shown by the mating calls of this group may be the result of complex selection pressures, where abiotic and behavioural factors have influenced the evolution of the vocalizations for optimum broadcast and maximum species-specificity.

The selection pressures on anuran vocalizations should not be underestimated in phylogenetic studies.

## 5 GENERAL ECOLOGY

### 5.1 Habitat selection and overlap

The positions in the habitat utilised by each species were characterized spatially into horizontal and vertical components. This method follows that of Heyer and Bellin (1973). I recognized the Lesotho plateau, the Drakensberg mountain slopes, forest, grassland and disturbed habitat categories. Horizontal divisions of each habitat were: in stream, in vlei, on bank, away from water, edge of pond, middle of pond. The following vertical divisions were recognized: under rocks, under bank, in vegetation, in water/on surface of soil, on rock, in hole, in rotten log, on vegetation above ground level, under plant debris, in rock cracks. More categories exist, but the species studied could be placed in a matrix using only those listed. Figure 28 shows the microhabitats utilized by each species in terms of this spatial analysis. Altogether 55 different microhabitats were recorded for the group. Figure 29 shows the number of habitats occupied and shared by each species.

	VLEI	STREAM	ON BANK	AWAY FROM WATER	MIDDLE DEEP POND	EDGE OF POND	
ALPINE		D V G	D				on Surface
				G			in Vegetation
		V					under bank
		D V	D V	Hy			under rocks
MOUNTAIN SLOPES		A		Hy		A Hy	on Surface
				G A	A	W	in Vegetation
		A					on rock
				G			in holes
		Hy	Hy	Hy			under rocks
FOREST		W	W A	A W		A	on Surface
		W A	W A	G		A	on rocks
				G			in rotting logs
GRASSLAND				Fa	Fu	Fa	on Vegetation
	G	A	Fu A G	A	Fu	Fu G A Fa	on Surface
						Fa	under debris
	G Fa	Fa	Fa	A	Fu	Fu A Fa	in Vegetation
			A				on rocks
		A	G			G	under bank
		V		Fu			under rocks
DISTURBED		G	G	G		A	on Surface
		G					in Vegetation
						G	in Cracks

Figure 28. Spatial analysis of the microhabitats occupied by each species. Abbreviations as in figure 9.

	TOTAL No. HABITATS OCCUPIED	TOTAL No. HABITATS SHARED	TOTAL No. HABITATS UNIQUE
A	19	9 (5)	10
D	4	3 (2)	1
Fu	7	3 (3)	4
V	5	3 (2)	2
Hy	6	1 (1)	5
W	6	4 (1)	2
Fa	8	3 (3)	5
G	17	5 (5)	12

Figure 29. Table showing the number of microhabitats shared by each species, the number of microhabitats occupied by each species which are not occupied by any other species in this group, and the total number of microhabitats which each species utilizes. Microhabitat categories from fig. 28. Number in brackets refers to the number of species of this group which share the habitat, e.g. angolensis shares nine habitats with five other members of this group. Abbreviations as in figure 9.

## 5.2 Predator-prey relationships

The group as a whole consume mainly terrestrial arthropods. Coleoptera and orthoptera were most common in 207 guts examined, although hemiptera, ants and spiders were frequently found. Some interesting food items were taken; caterpillars and millipedes (fuscigula), aquatic insect larvae (damselfly and coleoptera)(grayi) indicating that this species may feed in shallow water, gastropods (wageri and vertebralis), crabs (fuscigula and vertebralis). In the laboratory vertebralis would locate and consume small crabs (carapace 30mm wide) while underwater, which is a unique method of feeding in this group. Fuscigula apparently takes crabs along the water's edge where it commonly feeds.

R. temporaria in Europe are regarded as unspecialised terrestrial opportunistic feeders (Houston 1973, Blackith and Speight 1974) although there is one record of R. temporaria eating aquatic insect larvae (Smith 1949). Inger and Greenberg reported that three large species of Rana in Sarawak fed on the most abundant forest invertebrates in a particular



size range. They also feed on crabs which are abundant at night along the edges of most forest streams, and on smaller frogs (Inger and Greenberg 1966). R. cancrivora eats what is available including crabs and molluscs but does not feed in water (Elliott and Karunakaran 1974). I could find no records in the literature of other Rana species which feed underwater like vertebralis.

These frogs are apparently fed on by snakes, birds and other frogs. Otters are partial to these frogs in Natal (Rowe-Rowe 1977a,b). Vertebralis and fuscigula will consume smaller frogs without hesitation. I have seen a crab climbing up vegetation over a pool and eating the eggs of wageri deposited there. Many carnivorous insect larvae feed on tadpoles.

### 5.3 Breeding season and male calling sites

Figure 30 indicates the breeding activity of the eight species in the study area based on records of eggs, amplexant pairs and mating calls.

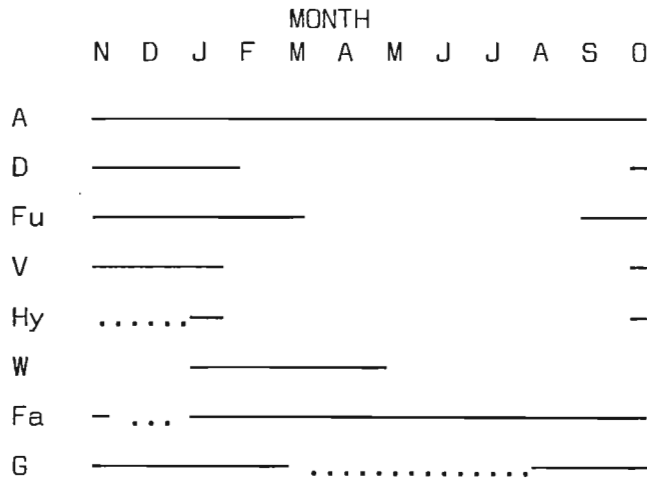


Figure 30. Seasonal breeding activity of the species. Solid line: known breeding, dotted line: presumed breeding. Species abbreviations as in figure 9.

Male calling sites are frequently species-specific in anurans (Bogert 1960). The following calling sites were utilized by males in this group: angolensis calls from the water's edge, or from a platform of vegetation just above water level. Species "D" may call some distance from water, or at the water's edge. Fuscigula typically calls from within clumps of

sedge-like plants in deep pools, often in the middle of large bodies of water. Vertebralis calls while only the head protrudes above water level, and from the top of rocks in the river. Hymenopus and wageri have not been heard calling in the field, but in the laboratory both called at the water's edge. Wager (1965) reported that wageri sometimes calls while submerged. Grayi calls from concealed sites under banks, in cracks, or hidden in dense vegetation. The calling sites are typically damp and shaded, and grayi commonly calls during the day and night. Fasciata calls from the base of grass in wet areas and will call commonly above ground level in vegetation (I have recorded fasciata calling up to one metre above ground). Males climb up shrubs and thick grass, and call from concealed sites in the vegetation. Fasciata are the most difficult of this group to locate while they are calling, unless the vegetation is sparse and they are compelled to call from ground level.

#### 5.4 Oviposition

Oviposition sites, numbers of eggs, sizes of egg capsules, and comparisons from the literature are given in fig. 31.

	OVIPOSITION SITES	NUMBERS OF EGGS	SIZE OF CAPSULE	NOTES
A	Shallow, calm water	400-500	5mm	Wager (1965) states thousands of eggs are laid
D	Shallow, calm water	150	5mm	Eggs are laid singly or in small clumps
Fu	Bottom of quiet pools	15000 (Wager)	3,5mm	
V	Attached to vegetation in quiet pools		5mm	
Hy	Attached to rock in mountain streams	200-500	9-10mm	Eggs laid in batches (7-15) or in one mass
W	Attached to vegetation in slow streams and pools	120-250	6mm	Wager records 20-30 eggs per batch
G	On land in damp sites and in water	250-350	4mm	
Fa	In shallow water		4mm	

Figure 31. Oviposition sites, size and numbers of eggs. Abbreviations as in figure 9. Numbers of fasciata and vertebralis eggs unknown.

### 5.5 Discussion

Ecologically the eight species show a broad similarity, although each possesses peculiar adaptations. No two species are ecologically exactly alike. Two trends are apparent, one is the trend from aquatic to semi-terrestrial breeding and the other is from a eurytopic state (adaptable to many different kinds of habitats) to a stenotopic state (restricted to special habitats).

The group adapted for stream breeding (vertebralis, hymenopus and wageri) attach their eggs to vegetation or to the rocky substrate, which is an adaptation to prevent the eggs from being washed away. An appropriate adaptation to the forest environment is shown by wageri which utilizes vegetation overhanging the breeding pools for egg attachment. In addition, this species only breeds during the dry winter or autumn when the pools have stabilized. If the water level should drop and the eggs become exposed to the air, they would be in no immediate danger of desiccation in the damp shaded forest habitat. By breeding after the wet season, wageri ensures that the eggs are not subjected to flooding, which is particularly disruptive in areas of steep slope when water action rolls boulders and scours out established vegetation.

Hymenopus appears to avoid floods by breeding when the rivers are low, and by attaching the eggs to a solid rock substrate. This species may also deposit eggs away from the stream in shallow pools. Vertebralis also avoids running water, ovipositing in quiet backwaters.

The next group is adapted for breeding in vleis and slow flowing streams, which is typical of the grassland habitat. The four species in this group (angolensis, fuscigula, D and fasciata) all lay eggs in shallow water, loosely spread on the bottom where they rapidly gather particles of sand and mud, soon becoming well camouflaged. The conditions under which oviposition occurs are relatively stable. The direct result of a temporally uniform environment is that the frogs are able to breed throughout the year. Exceptions to this are habitats which are snowed in in winter, and areas where free water is not available for a period preceeding the first summer rains.

The last group has only one member, grayi, which is characterised by frequently ovipositing out of water. Females select cool damp sites, often under leaves or other cover. Not all grayi eggs are laid on land, however. It is not uncommon to find an egg mass which

is wholly or partly under water. This has been suggested as evidence for an actively evolving species (Van Dijk, personal communication), but I believe that the tendency for terrestrial breeding in grayi is not a new, derived state, but rather an ancestral condition which evolved under forest conditions where the eggs were protected by damp shady environments.

The transition to terrestrial breeding in amphibians has been correlated with rapidly flowing montane streams (Goin and Goin 1962), larval predation (Poynton 1964b) and improved oxygen supply with increased temperature for faster development (Van Dijk 1971b). Leptodactylids in South America (Heyer 1969) and Australia (Martin 1970) have adapted to a range of conditions and breed in aquatic, terrestrial and many intermediate situations. It seems that the group examined in the present study have also adapted to breed in the existing range of environments within their limitations as "river" frogs.

The second pattern of eurytropy vs stenotopy is clearcut: from figure 29 it can be seen that "D", fuscigula, vertebralis, hymenopus, wageri and fasciata are confined to relatively few microhabitats (4-8) while grayi and angolensis occupy a wide range of microhabitats (17 and 19 respectively) sharing many with the first group. Ecologically grayi and angolensis represent good colonizing species, while the rest would tend to remain in their special habitats. The influence of eurytropy and stenotopy on the distribution of these species will be examined later.

## 6 COLOUR PATTERN ANALYSIS

### 6.1 Morphs recognized

A catalogue of colour morphs was started with the first few specimens caught. All colour patterns were painted from life so that subsequent captures of animals with similar patterns could be recorded. No attempt was made to reproduce the colour patterns exactly, but rather to categorize the specimens into broad pattern types. As each new morph was recognized as such by comparison with the catalogue, it was in turn painted. Altogether 26 different morphs were recognized.

## 6.2 Results and discussion

The morphs recognized from the study area are shown in figure 32. I am aware that other colour patterns are found in populations in other parts of the country (for example the broad red vertebral stripe common in populations of grayi from the Transvaal), but this study is limited to the defined area (fig. 1).

In figure 32 it will be noticed that some of the patterns are shared by wageri and angolensis, some by grayi and angolensis, some by fuscigula and angolensis, and some by "D" and angolensis. One pattern is shared by fuscigula, angolensis and grayi, and another two are shared by "D" angolensis and fuscigula.

When considering these patterns a question springs to mind: What influences the number of morphs – what is the advantage of a large or small number of colour morphs?

The number of colour morphs possessed by a species is presumably a function of the range of habitats occupied – a species occupying a large number of habitats with different background colours, shapes and lighting, would be expected to have morphs suited to a wide range of background conditions. Alternatively the number of morphs may be an anti-predator device by making visual predation more difficult by varying the prey image independently of the background. If this were the case I would expect a species with a large number of morphs to be correspondingly more successful than a species with few colour morphs. In both cases the more successful species (in terms of range of habitats occupied) would be expected to possess more morphs than less successful species.

The number of microhabitats occupied (fig. 28) can be compared to the number of colour morphs possessed by each species (fig. 32). This comparison is presented as figure 33. Angolensis and grayi which have the largest number of colour morphs are also the two eurytopic species. It seems that there is a relationship between the ability to colonize and the number of colour morphs in a species. The relatively high number of colour morphs of "D" (one less than grayi) when compared to the low number of microhabitats occupied may be explained by the uniformity of the habitat reducing the possible microhabitats, while it may be a relic of pre-angolensis stock, which still possesses the range of colour morphs.

Colour polymorphism in frogs has been the subject of previous genetic studies (Matthews 1971, Fishbeck and Underhill 1971). The



Figure 32. Illustrations of the colour morphs recorded in this study.

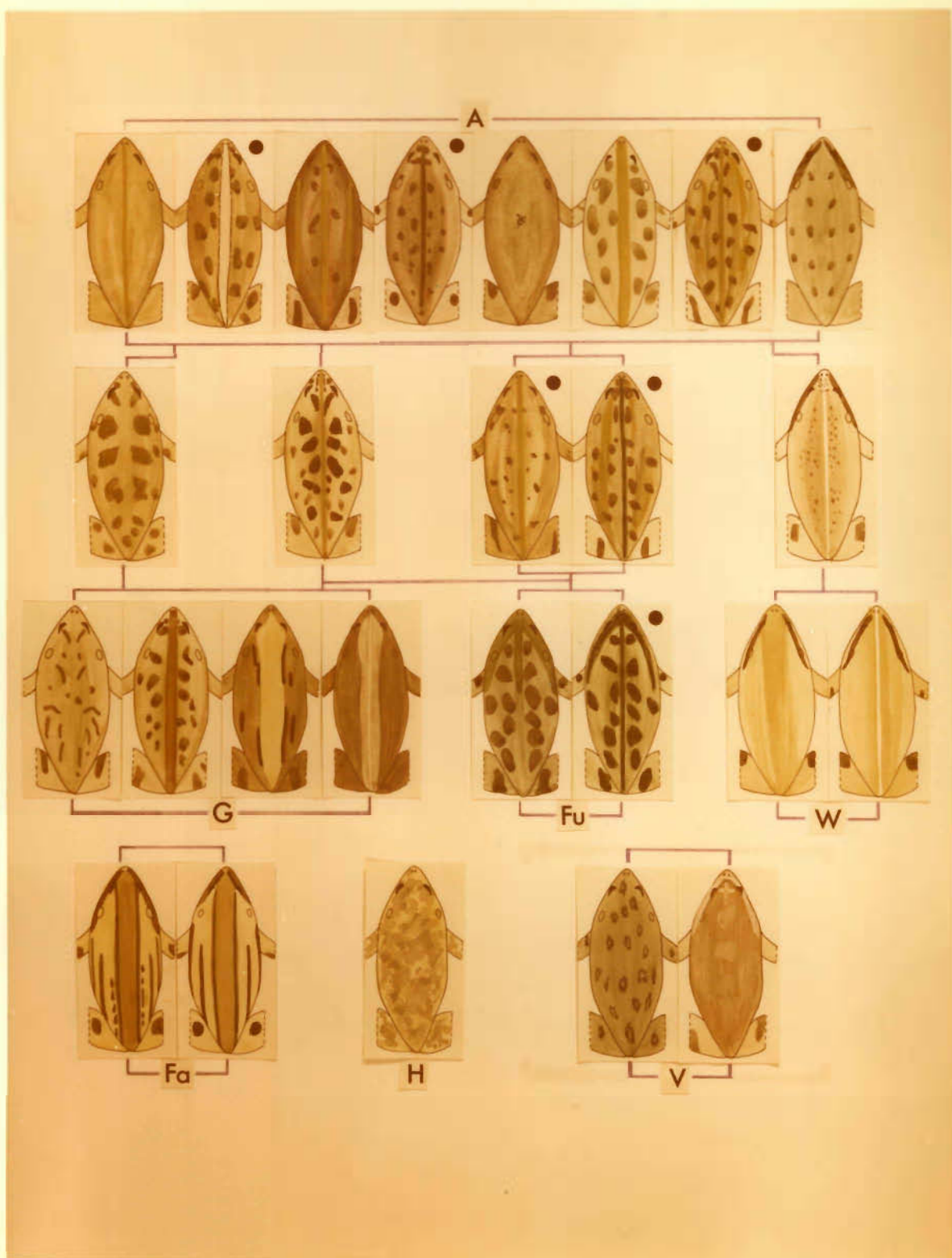
Top row: Colour morphs found in angolensis.

Second row: Colour morphs found in angolensis, but shared with grayi (left two); fuscigula (middle three); and wageri (extreme right).

Third row: Colour morphs found in grayi (left four); fuscigula (next two) and wageri (right two).

Bottom row: Colour morphs found in fasciata (left two); hymenopus (middle one) and vertebralis (right two).

Spots: The six colour morphs with a black spot to the right of the illustration are found in form "D".



relationship between area occupied and number of colour polymorphs has been demonstrated previously in molluscs (Rex 1972). The frog genus Phrynobatrachus was examined for pattern polymorphism by Stewart (1974). She found that the pan-African P. natalensis shows the most pattern stability probably due to nocturnalism. Savanna species all look alike and have similar pattern morphs, while forest species of Phrynobatrachus look very different to savanna species and have a different set of colour morphs. Stewart believes that the patterns in the genus probably function as protective camouflage against predators with keen vision. In the group of species I studied only wageri and fasciata have unique patterns, while all the other species have broadly similar patterns. Wageri and fasciata are confined to special habitats (forest and vleis) while the other species (except hymenopus) all live in savanna/alpine grassland habitats. The maintenance of colour polymorphism is presumably a function of differential predation in the grassland species. However, more selection pressures may be operating, as the colour patterns in North American Acris are maintained by three pressures: Visual selective predation, climatic selection and the physiological resistance of particular morphs (Nevo 1973).

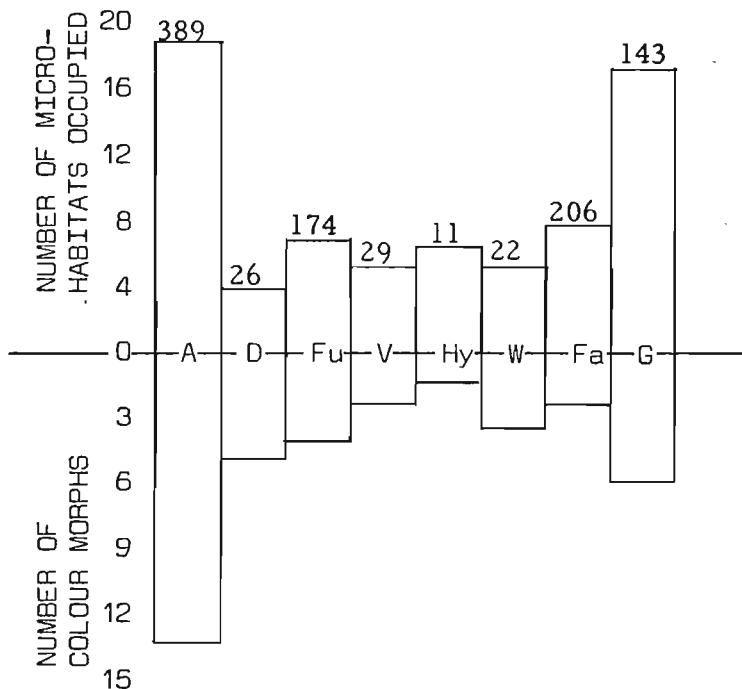


Figure 33. Comparison of the number of colour morphs and number of microhabitats occupied by each species. Abbreviations as in fig. 9. Number on top of column is sample size.

## 7 DISTRIBUTION

### 7.1 Distribution within the study area

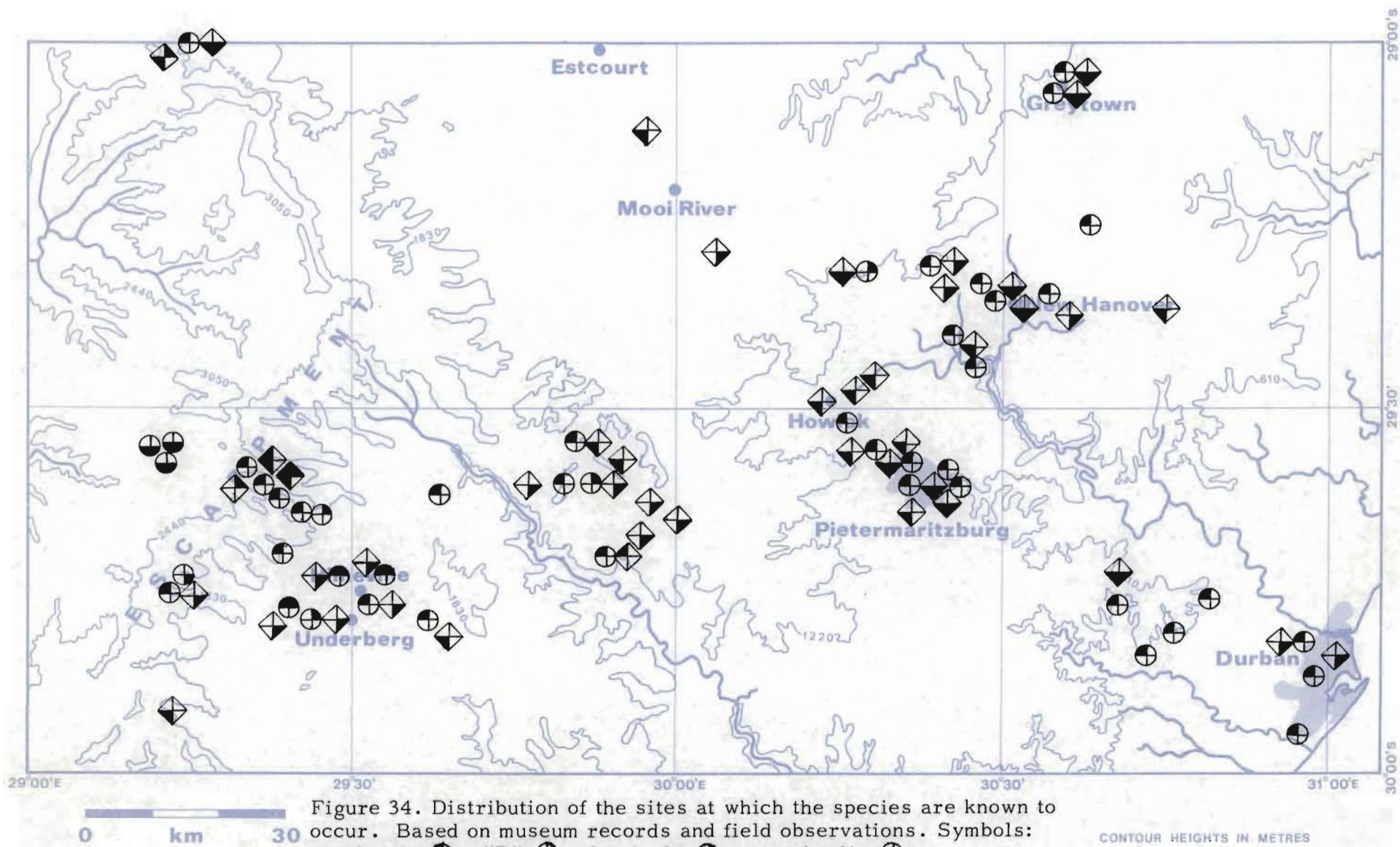
The distribution of sites at which the species is known to occur is shown in figure 34. Vertebralis and "D" are confined to the Lesotho plateau, with the exception of a population of vertebralis in the Umzimkulu river at the foot of the Drakensberg (Bush 1952, and confirmed in the present study). Hymenopus is restricted to the slopes of the Drakensberg and the mountains of Lesotho. Wageri has been found on the lower slopes of the Drakensberg and in streams in forest patches throughout Natal. The Natal uplands are the only area where fuscigula occurs in the study area, while angolensis is widely distributed from the slopes of the Drakensberg to the coast. The remaining two species, fasciata and grayi, also occur throughout the area, although fasciata does not leave the flat river valleys at the foot of the Drakensberg, while grayi extends on to the Lesotho plateau.

### 7.2 Correlation with slope and altitude

Figure 35 is an analysis of the slopes at the sites where frogs were collected. The altitudes at which the various species were collected is shown in figure 36. Altitude was read off the 1:50 000 series maps of South Africa, while slope was calculated as mean slope along a 0,75 km transect centered on the collecting site. Mean slope reflects the general topography of the environment, and correlates with speed of river flow and vegetation.

From figs 35 and 36 it can be seen that fuscigula, vertebralis, "D" and fasciata are found on relatively flat sites. "D" and vertebralis are found on top of the Drakensberg, while fuscigula occurs between 1000-1500m. Fasciata occurs throughout the area from the coast to the foothills of the Drakensberg. Hymenopus is restricted to high altitudes, while wageri is restricted to areas of steep slope at higher altitudes. The remaining two species, grayi and angolensis, are found on all slopes and at all altitudes.







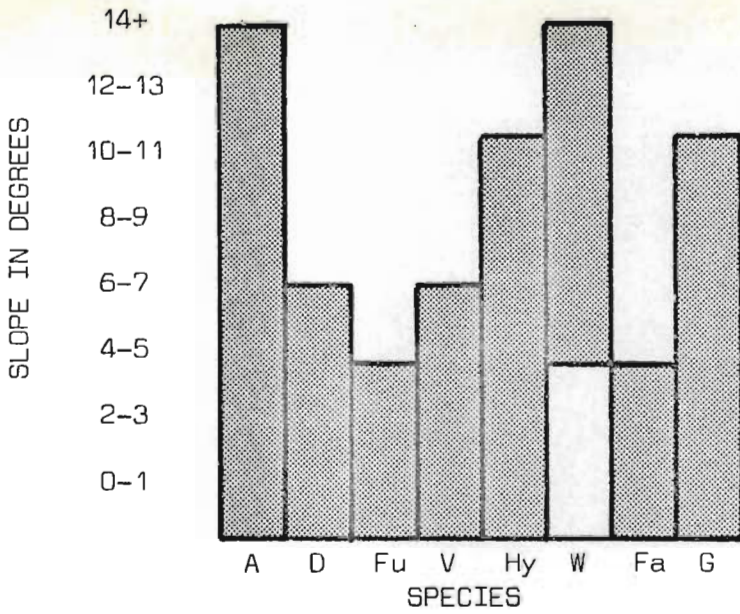


Figure 35. Analysis of the angles of slope of the sites at which specimens were collected. Abbreviations of species as in figure 9.

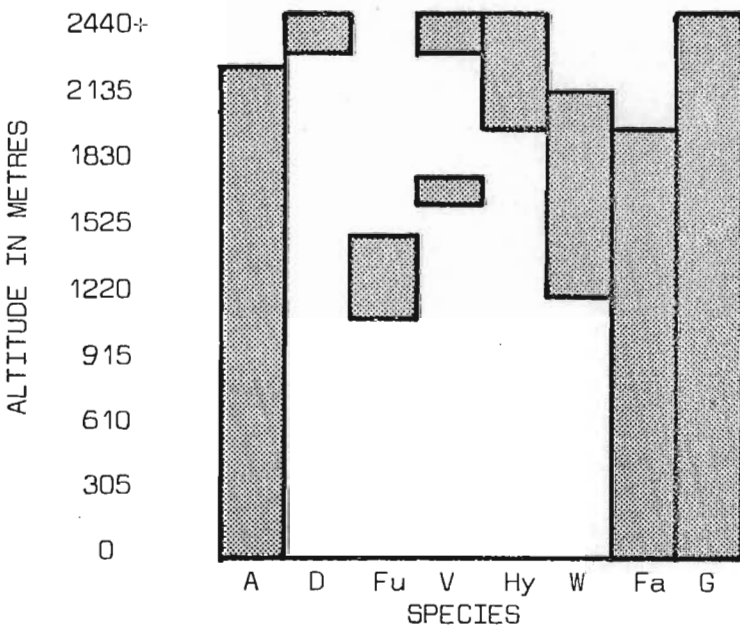


Figure 36. Analysis of the altitudes at which specimens were collected. Abbreviations as in figure 9.

### 7.3 Correlation with geology

Figure 37 shows the geology of the study area. "D" and vertebralis are confined to the Drakensberg series (with the exception of the population of vertebralis in the Umzimkulu river at the Holkrans-Beaufort contact). Hymenopus occurs on the Holkrans and Drakensberg series, while the other species occur widely, only angolensis and grayi reaching the Holkrans, and only grayi occurring right up to the Drakensberg series.

The Drakensberg basalts are the dominant scenic element in Natal, as their relative hardness during many cycles of uplifting in the geological history of the area has led to the formation of the characteristic Drakensberg escarpment (King 1972). The significance of the geology in a study of the distribution of these frogs is that the resistant basalts have produced a high altitude plateau and a steep escarpment with fast-flowing streams. The formations over the rest of the area are predominantly sedimentary, and have weathered to gentle undulating uplands and deeply incised valleys, with sandstone and dolerite capped scarps. The distribution of the species studied correlates with the topography of the area which is in turn a direct result of geological processes.

### 7.4 Correlation with vegetation

The present vegetation of the area is shown in figure 38. The dominant floristic elements are the Themeda-Festuca Alpine veld, the Ngongoni- and Sourvelds, and the forests of the coastal and valley bushveld (Acocks 1975). Killick (1963) gives an account of the vegetation of the Cathedral Peak area, which is on the northern edge of the study area.

Comparison of the distribution of the species (fig. 34) with the vegetation of the area shows that wageri is confined to forest (valley bushveld) except on the Drakensberg slopes where it occurs in wooded areas. Hymenopus, "D" and vertebralis are found within the alpine veld, while fuscigula occurs mainly in the area of Highland- and Dohne Sourveld. Fasciata occurs throughout the area below the escarpment in wet grassy situations. Grayi and angolensis occur widely in all the vegetation types, although angolensis does not breed in forests or occur beyond the margins of the alpine veld, as grayi does.

Acocks (1975) suggested that in the recent past (1400 AD) Natal was covered in forest and scrubforest, with grassveld on top of the



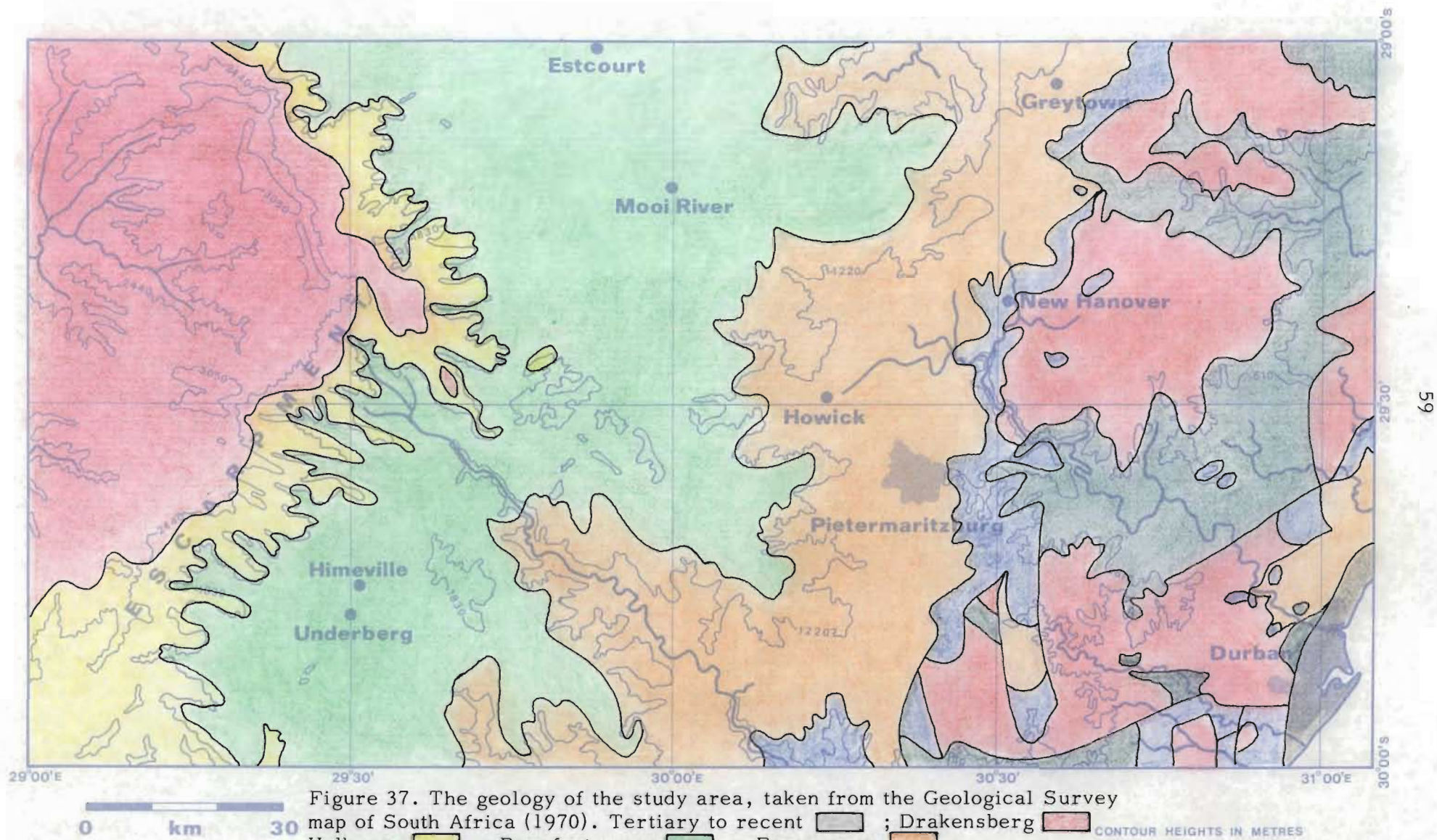


Figure 37. The geology of the study area, taken from the Geological Survey map of South Africa (1970). Tertiary to recent  ; Drakensberg  ; Holkrans  ; Beaufort group  ; Ecca group  ; Dwyka group  ; Table Mountain group  ; Namaqua-Natal mobile belt rocks  .



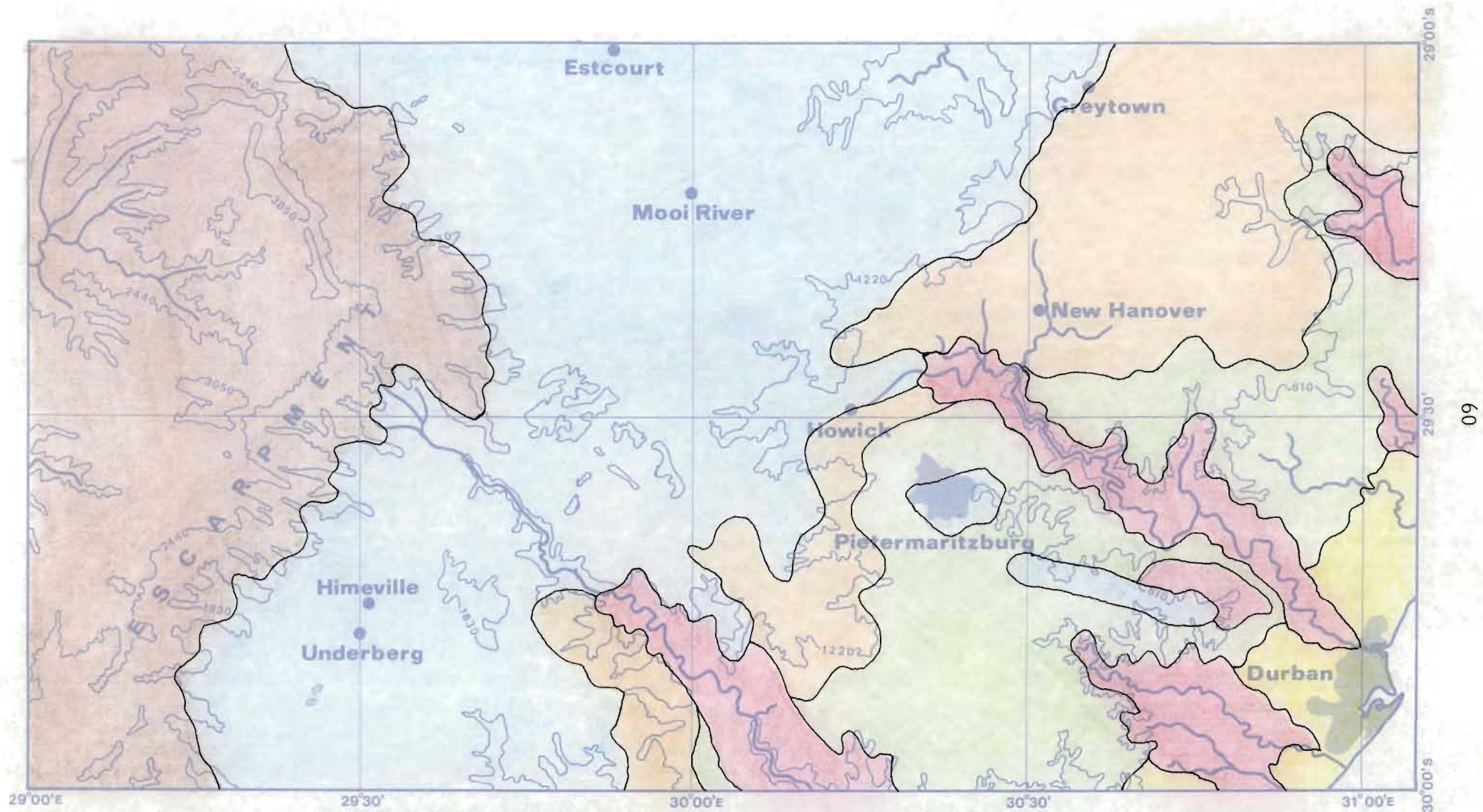


Figure 38. The vegetation of the study area, after Acocks (1975):

Coastal forest  ; Ngongoni veld  ; Valley bushveld  ;  
 Highland and Dohne Sourveld  ; Ngongoni veld of Natal Mist Belt  ;  
 Themeda-Festuca alpine veld  .

CONTOUR HEIGHTS IN METRES

Drakensberg. If this were so, it could explain the present disjunct distribution of wageri in forest patches which were once joined. It also suggests that the grassland species have expanded their ranges recently and may still be actively colonizing. Both fuscigula and angolensis are savanna species, and would have been isolated from each other by a forest barrier, with fuscigula at the Cape, and angolensis to the north. They are presumably now in competition where they are sympatric. The contraction of the forest may also explain the distribution of hymenopus which now occurs as a relict where forests once stood on the mountain slopes. This species is presently found near wooded areas on the escarpment.

#### 7.5 Southern African distribution

The most recent literature on the distribution of these frogs (Van Dijk 1977) was used to compile a map of species density in southern Africa (fig. 39). Only the broad outlines of the distribution of each species were used to draw the map, consequently it is not accurate for detailed locality studies, but it does indicate a rough picture. The reader is referred to Van Dijk (1977) for details of species distribution.

Figure 39 shows that no members of the group occur in the dry interior of the Kalahari, Namib and Karroo regions, although fuscigula and grayi are found on the west coast up to Namaqualand. The isolated population of fuscigula in springs in the Naukluft Mountains of South West Africa suggests a relict from wetter times. However, to my knowledge the area south of Naukluft has not been well collected and it would not be surprising to find populations of fuscigula along the length of the Fish river, joining the Naukluft-Mariental populations (Channing and Van Dijk 1976) to the South African populations. The record for grayi from Mariental (discussed by Van Dijk 1971a, 1977) may be based on a misidentification of juvenile fuscigula. The specimens in question consist of a number of metamorphosing tadpoles and juvenile frogs with tails. The only reliable character for species identification at that stage is the degree of webbing. Fig. 23 shows that it is possible for fuscigula with two phalanges free of webbing to be confused with grayi. The lack of any adult grayi despite collecting at Mariental by various people suggests that grayi does not breed in the area and probably does not occur there at all. When Poynton included this record from Mariental on the distribution map of grayi (1964a), fuscigula had not yet been collected there.



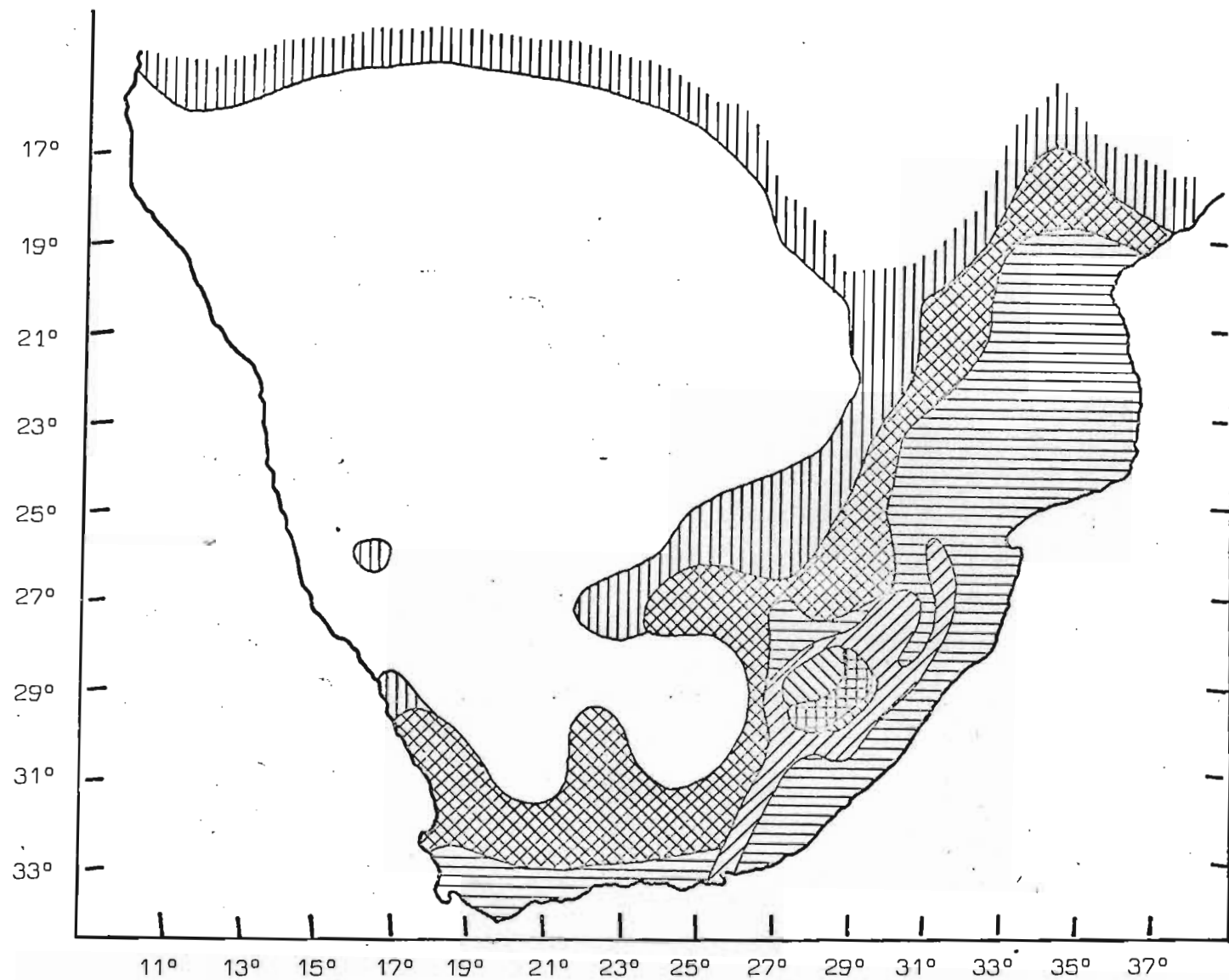


Figure 39. Map of the species density of the forms studied. Species density is highest around the Drakensberg, decreasing radially. Based on Van Dijk (1977). Symbols: 6spp; 5spp; 4spp; 3spp; 2spp; 1sp.

Figure 39 shows the highest species density in the Drakensberg area, becoming progressively less dense moving away from Lesotho. This pattern suggests a centre of speciation (Udvardy 1969), which is consistent with the results presented earlier.

However, the pattern shown may be caused by the availability of habitats in areas of high relief, with no significance in terms of the origin of the group. If the pattern were really random, I would expect a random clumping of species, possibly on the Cape mountains and on the Drakensberg. As the pattern is remarkably consistent, it suggests that the Drakensberg has served as a centre of refuge and speciation for the group in past times. The relationships of the southern African species to other species in Africa and world-wide, remains an intriguing problem.

#### 7.6 Discussion

The factors controlling animal distribution relate to the ability of the species to disperse, the availability of suitable habitat and the time which the species has had to disperse. The study group consists of frogs which are highly vagile, limited only by extremes of aridity. This is evident when we consider that no member of the group occurs within the arid centre of the country. There are certain relationships in the distribution of these species with altitude, slope, geology and vegetation. It would be convenient to prove causal relationships, but in the absence of experimental evidence (which is not possible) it must suffice to merely record the correlations and suggest explanations. Hypotheses based on descriptive studies need be no less valid than those based on empirical studies.

One of the easiest environmental variables to measure is temperature, and it is not surprising that a lot of debate has centered on temperature as a key factor or indicator (direct or indirect) in the distribution of anura in southern Africa (Poynton 1960, 1964a, 1972; Van Dijk 1971a; Stuckenberg 1969; Poynton and Bass 1970). Rainfall (Van Dijk 1971a) has also been suggested as a factor influencing the distribution of anura. Detailed microclimatic requirements of southern African anura are lacking, and long term weather recording stations are few, hardly ever occurring at the critical boundaries of frog distribution. These two limitations preclude a correlation of distribution with climate in the study area.

Past climatic changes have been suggested as factors influencing the distribution of animals in southern Africa (Poynton 1964a, Brain and Meester 1965, Channing 1976). Available paleoclimatic evidence indicates two cool periods in the late Pleistocene possibly of 5,5 °C and 9 °C, when total precipitation may have been higher than present. The temperature of the Natal Drakensberg may have been down to -8 °C with possible glaciation (Harper 1969). Fitzpatrick (in press) has shown that permafrost was present in the Drakensberg during the late Pleistocene. The last hypothermal was probably 12600-9650 BP (Van Zinderen Bakker 1969). Rainfall during the Pleistocene was set at about 60% of present for interpluvials and 140% of present for pluvials by Brain (1958). Paleoclimatic changes in Africa have been reviewed by Livingstone (1975).

Past climatic changes would be related to past vegetational changes like those indicated by Cooke (1962). Apart from the floristic evidence which led Acocks (1975) to suggest that Natal was covered in forest until recently, Willcox (1974) discusses evidence from Middle Stone Age sites for the presence of forest in Natal above 4000 feet (1200m).

The significance of past climatic changes on anuran distribution may be judged from the report by Blair (1965) which suggested that most present patterns of amphibian distribution in the United States are due to Pleistocene events. Glaciation has displaced some species and pluvials have isolated others. Anuran relicts in deserts represent remnants of pluvial stage distributions. I have suggested that the occurrence of fuscigula in South West Africa may be explained in a similar fashion.

If the forests of Natal have recently been replaced by grassland as Acocks suggests, field studies are needed to determine if the ranges of the savanna species are increasing, and the rate of such expansion.

The pattern of species density in this group (fig.39) is not unique. Barbus anoplus and B. pallidus are endemic to southern Africa and are centered on the Lesotho area (Jubb and Farquharson 1965). Studies on North American mammals have showed that high species density is associated with high topographical relief (Wilson 1974). Wilson showed a correlation with AE (actual evapotranspiration) and distribution. A later study on Texas amphibians showed that species density of frogs increases with increasing relief, probably due to greater numbers of habitat types available (Rogers 1976). Rogers also showed that altitude per se has a negative effect on frog species density. The number of species per genus of frogs increases with increasing mean annual precipitation and with an

increase in altitude (Rogers 1976). The species density of the frogs in my study shows a correlation with relief and altitude, but precipitation is lower in Lesotho than at the coast and areas in the Cape, so it appears that precipitation is not responsible for the high species density centered on the Drakensberg. Past precipitation patterns would have determined the distribution, and these patterns may not be the same as are found now. The high mountains may have served as a cool moist refuge for anurans during interpluvials and as centres from which they radiated during pluvials.

The influence of humans on the distribution of these frogs is minimal in the study area, with the possible exception of wageri, which has no doubt disappeared along with much indigenous forest. Farm dam edges have provided new area which have been occupied by the savanna species, while many ditches and gardens are occupied by grayi. Timber plantations in the midlands are devoid of anurans, except some Bufo spp.

The group of frogs studied is in no danger from humans, in contrast to the situation in densely populated areas like the British Isles, where populations of R. temporaria and Bufo bufo have recently become markedly reduced due to the effect of agricultural expansion (Cooke 1972).

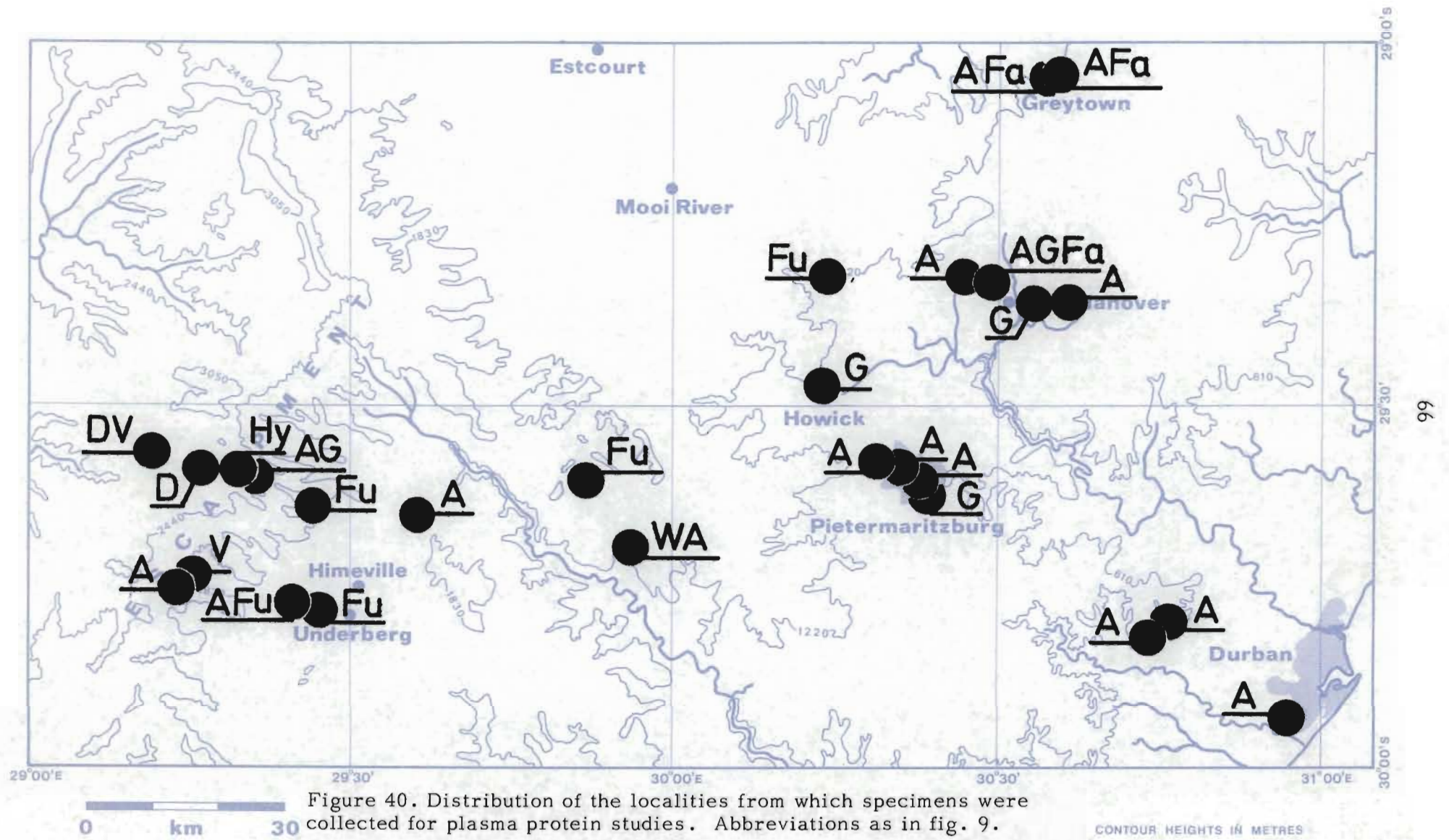
## 8 PLASMA PROTEINS

The electrophoretic analysis of proteins is an established method of detecting evolutionary affinities (Hubby and Throckmorton 1965). There has been an increase in the number of anuran blood protein studies reported in the literature (e.g. Cei 1972a,b; Guttman 1969; Rogers 1973; Platz 1976; Tunner and Dobrowski 1976). The reader is referred to Avise (1974) for a critical view of the systematic value of electrophoretic data.

### 8.1 Methods

Disc electrophoresis (Davis 1964) was used to separate plasma proteins. Blood samples were removed from the heart with microhaematocrit tubes, and spun down in a clinical centrifuge. The plasma was stored in sealed microhaematocrit tubes at  $-21^{\circ}\text{C}$  until used. After electrophoresis the gels were stained for specific proteins: esterases, phosphatases and lactate dehydrogenase (LDH). Detailed plasma sampling technique, methods of electrophoresis and enzyme identification procedure will be found in the appendix.







## 8.2 Results

The localities from which samples were obtained are shown in figure 40. These results are based on 306 sample gels. Due to the scarcity of some species large samples from each species were not tested. Consequently gene frequencies could not be calculated, but the phenotypes and total alleles expressed in each species were examined. The phenotypes for each of the three proteins for each species are compared in figures 41, 42 and 43.

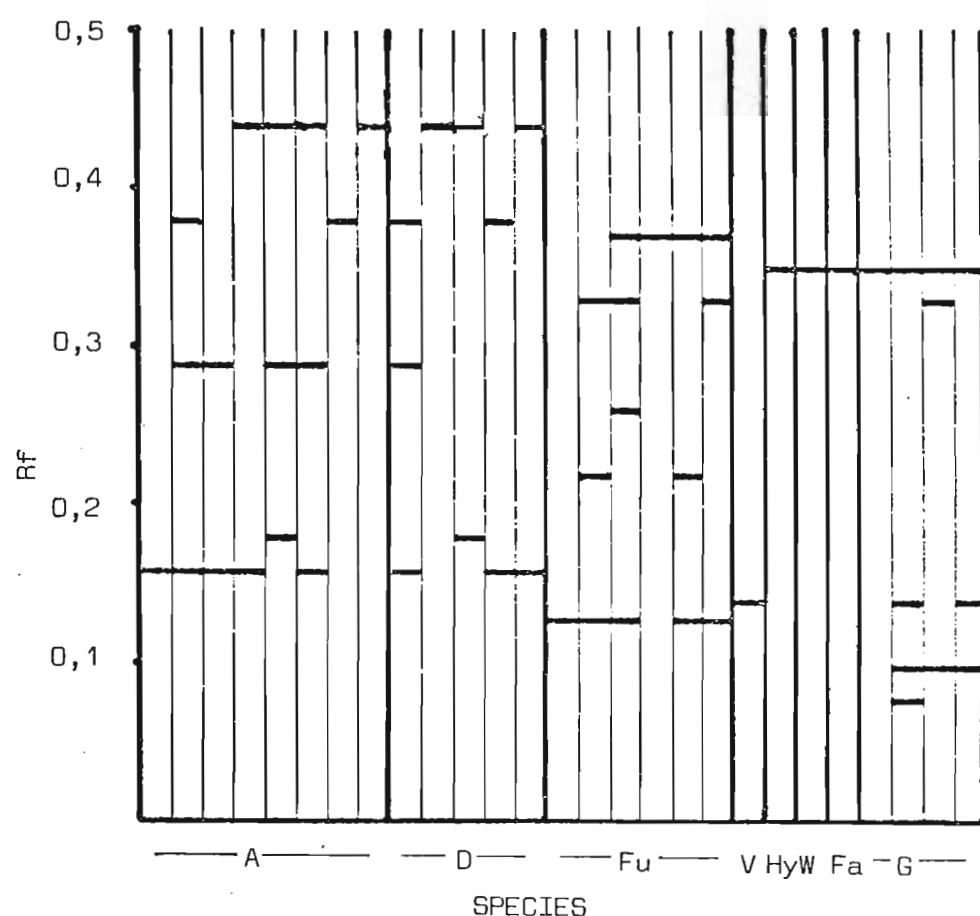


Figure 41. Phenotypes of phosphatase alleles found in the species studied. Rf=relative mobility of the protein fraction relative to the front (bromophenol blue). Each column represents a phenotype. Species abbreviations as in figure 9.

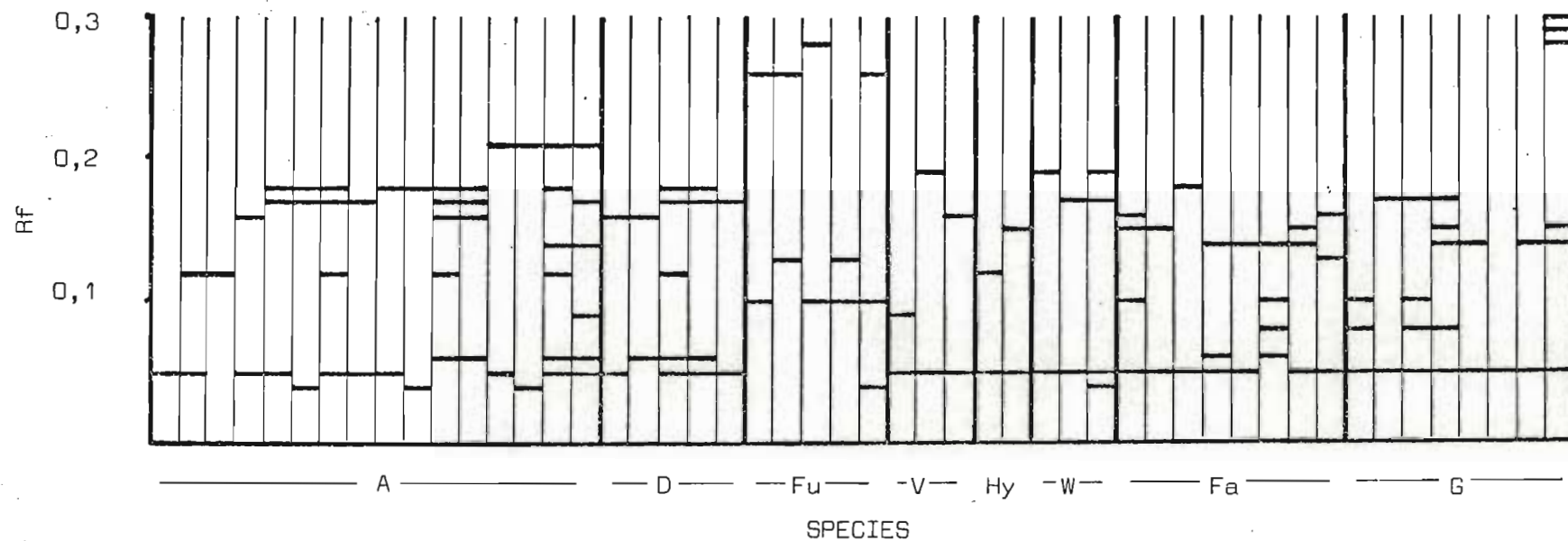
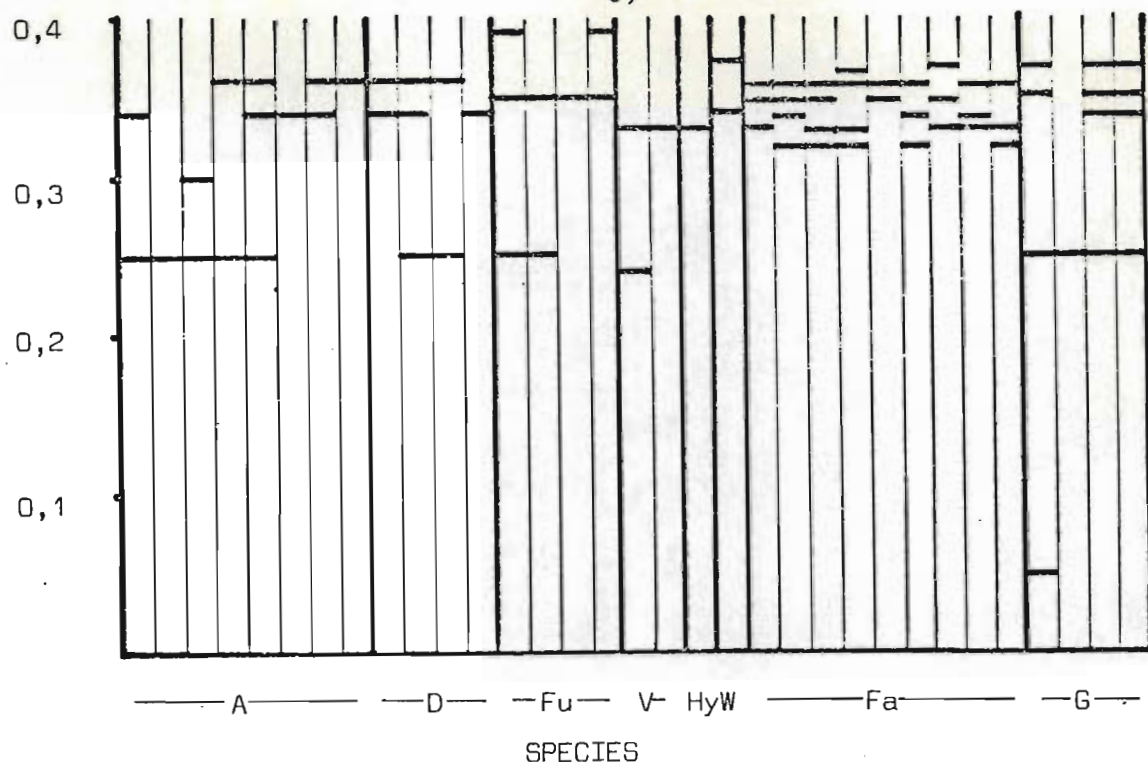


Figure 42. Phenotypes of the LDH alleles detected in the species studied.  
 Rf= Mobility of the protein fractions relative to the front (bromophenol blue). Each column represents a phenotype. Species abbreviations as in figure 9.



See fig. 42 for explanation of Rf and interpretation.  
 Figure 43. Phenotypes of esterase alleles detected in the species studied.  
 Explanations of species abbreviations as in figure 9.

Guttman (1973) points out the pitfalls of comparing the positions of protein bands separated electrophoretically to determine degrees of relationship. Environmental factors may cause two unrelated species to appear more similar than two closely related species. With this caution in mind, the positions of the isozymes for each of the three proteins were compared using his expression for percentage affinity (Guttman 1973):

$$P_{ab} = \frac{(\text{sum shared bands})100}{\text{sum all bands}}$$

where  $P_{ab}$  is the percentage affinity of species a and b. Comparisons were made between the total isozyme complement known for each species.

All the phenotypes could not be included, as all the possible phenotypes were not known, therefore the percentage affinity as calculated is an underestimation. The following figures (44, 45, 46) give the percentage affinities of all the species pairs for each protein (100 is perfect similarity, 0 is complete mismatching). To facilitate comparison, a fourth table of mean  $P_{ab}$  values was computed, where

$$\bar{P}_{ab} = \frac{P_{ab}}{n}$$

The table of mean values is given in figure 47.

	G	Fa	W	Hy	V	Fu	D
A	0	0	0	0	0	0	100
D	0	0	0	0	0	0	
Fu	22	0	0	0	33		
V	40	0	0	0			
Hy	40	100	100				
W	40	100					
Fa	40						

Figure 44. Table of  $P_{ab}$  values for each species pair, comparing the positions of all the isozymes known in each species for phosphatase. Abbreviations as in figure 9.

	G	Fa	W	Hy	V	Fu	D
A	22	44	0	40	33	29	86
D	25	50	0	50	40	33	
Fu	50	25	0	0	0		
V	0	29	0	67			
Hy	0	33	0				
W	57	29					
Fa	40						

Figure 45. Table of  $P_{ab}$  values for each species pair for esterase. The positions of all the esterase alleles known to occur in the species were compared. Abbreviations for species as in figure 9.

	G	Fa	W	Hy	V	Fu	D
A	32	53	43	31	43	13	82
D	25	50	35	40	36	0	
Fu	29	29	22	0	0		
V	15	31	25	29			
Hy	33	33	29				
W	56	0					
Fa	56						

Figure 46. Table of  $P_{ab}$  values for each species pair for LDH. The positions of all the LDH alleles known to occur in the species were compared. Abbreviations for species as in figure 9.

	G	Fa	W	Hy	V	Fu	D
A	18	32	14	24	25	14	89
D	17	33	12	30	25	11	
Fu	34	18	7	0	11		
V	18	20	8	32			
Hy	24	55	43				
W	51	43					
Fa	45						

Figure 47. Table of mean  $P_{ab}$  values computed by averaging the values for figs 44, 45 and 46. Abbreviations as in figure 9.



The phosphatase alleles (fig. 44) have identical mobilities in the following species pairs: angolensis-"D"; hymenopus-wageri; and wageri-fasciata. Hymenopus, wageri, fasciata and grayi all possess an allele with a common mobility, but as grayi possesses more alleles in total, this species shows a low  $P_{ab}$  when compared to the other three. The esterase alleles (fig. 43) show that all the species possess similar phenotypes, although fasciata displays a large number of alleles with similar mobilities. The three highest  $P_{ab}$  values are found in the angolensis-"D"; vertebralis-hymenopus; and wageri-grayi species pairs (figure 45). LDH alleles have the most variation in mobilities, and the largest number of alleles in each phenotype. The four highest  $P_{ab}$  values show that angolensis-"D", wageri-grayi, fasciata-grayi and fasciata-angolensis are the most similar pairs (fig. 46).

Values above 40% in the table of mean  $P_{ab}$  values (fig. 47) indicate that, overall, the most similar species pairs are angolensis-"D", hymenopus-fasciata, hymenopus-wageri, wageri-grayi, wageri-fasciata and grayi-fasciata.

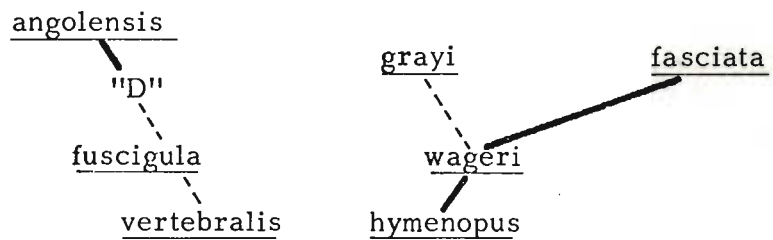
### 8.3 Discussion

The interpretation of patterns of plasma proteins needs to be approached cautiously. In studies where different alleles can be detected, the differences between individuals are exaggerated. Electrophoresis separates proteins of different molecular weights and charge. Most multiple enzyme forms do not involve differences of charge and are therefore likely to remain undetected by electrophoresis (Masters and Holmes 1972). If there is much phenotypic variation in a species, however caused, it is unlikely that all the phenotypes will be detected. The problem of determining the relationships between species is further complicated by the influence of both environmental and phylogenetic factors (Guttman 1973) on plasma proteins. Electrophoresis gives a "fine-grained" picture of the individuals of a species, yet measures of similarity like  $P_{ab}$  tend to give a "coarse-grained" picture, as they tend to underestimate relationships by not taking into account the degree of mismatching of isozymes. Two individuals with only one allele each, whether the alleles are separated by 1 or 10 Rf units would give  $P_{ab}$  values of 0. Yet it is apparent that a separation of only 1 Rf unit would indicate a similarity between the two species which would be absent if their alleles were 10 Rf units apart.

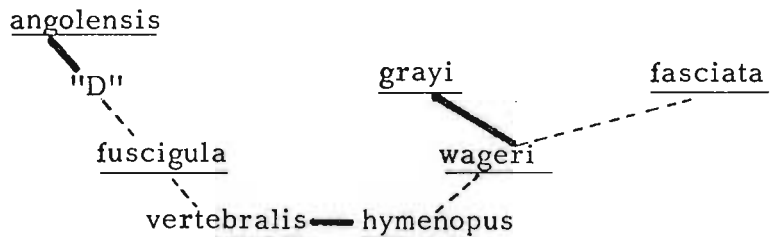
Despite these difficulties, the congruence between morphological and allozyme data in phylogenetic studies has been demonstrated by Mickevich and Johnson (1976). Different isozymes perform different physiological functions which may reflect both the selection pressure in particular environments, and the degree of divergence of the species from the ancestral stock (Masters and Holmes 1972).

High  $P_{ab}$  values as determined in this study may reflect similarity in both environmental and phylogenetic histories. Inserting the highest values (see results) for each enzyme, in the phenogram used before, gives the following (basic phenogram is dotted, high  $P_{ab}$  values are represented by solid lines):

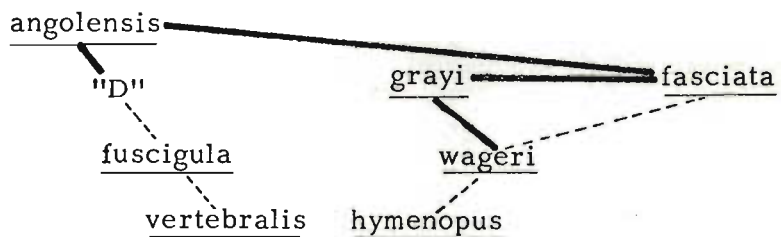
Phosphatase



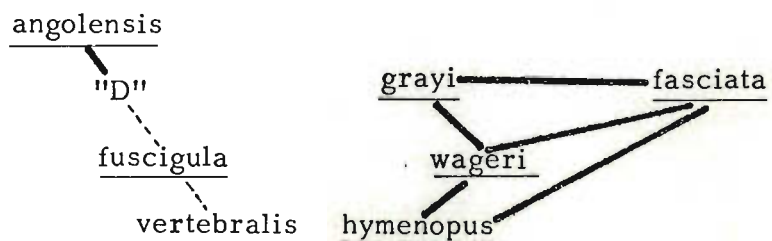
Esterase



LDH



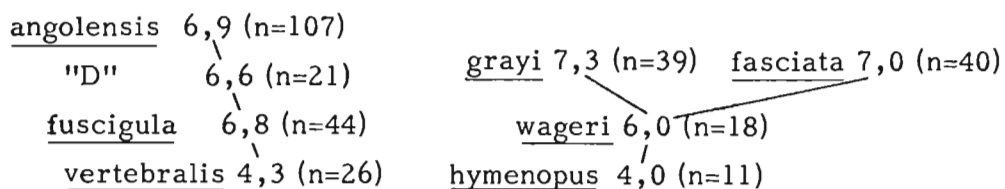
mean  $P_{ab}$



Except for two points, the diagrams above are in agreement with the phenogram. Fasciata and angolensis are shown to possess similar LDH alleles, and fuscigula does not appear to be closely related to any of the other species, although it does show a low degree of relationship to all the species except hymenopus (fig. 47).

The similarities of angolensis and fasciata may be caused by exposure to similar selection pressures in the grassland habitat, and not by a close phylogenetic relationship. The dissimilarities of fuscigula seem to be partly an artifact caused by the use of  $P_{ab}$ , an index which underestimates the similarity of species pairs, as inspection of the phenotypes for phosphatase and esterase (figs 41 and 43) shows. Fuscigula is similar to both "D", angolensis and vertebralis. This lack of close protein similarity of fuscigula and the other species may be due to the fact that fuscigula was isolated at the Cape during an interpluvial (by aridity) or by a forest barrier (see 9.1). Studies on the genus Kassina in southern Africa have suggested that K. wealei was isolated at the Cape, resulting in the evolution of striking differences in mating call structure compared to the species to the north (Channing 1976). Similar isolation of fuscigula could have led to the evolution of slightly different isozymes under different selection pressures.

Another way of interpreting the isozyme data is to examine the numbers of isozymes expressed in each individual, with the premise that the more specialized the species, the more additional isozymes will be present. This assumes that possession of many isozymes is a derived state while the ancestral condition was expression of a minimum number of alleles. When the mean number of alleles expressed by each species is compared on the phenogram used before, the following pattern appears:



Again fuscigula is the odd species, with a slightly elevated isozyme count. It is possible that selection has favoured the development of isozymes in response to the range of habitats occupied by ancestral "fuscigula". The phenogram may need revision, but this is unlikely as so much previous information on fuscigula fits so well. When compared with fig. 33, the



above pattern indicates that the widespread and colonizing species (angolensis and grayi) have a larger number of isozymes present in their plasma than those species living in restricted habitats

## 9 PHYLOGENY AND SYSTEMATICS

### 9.1 Past events

This chapter is a synthesis based on the evidence presented in the previous chapters. The speculations which follow will hopefully lead to a better understanding of the group as a whole, and point out areas of future research. (Fig. 47b summarizes this section)

The ancestral form from which all these species derived was probably a large frog adapted for cold moist conditions, and may have thrived on the margins of glaciers and similar situations. The mating call of this frog was probably a simple pulsed vocalization at low frequency. This frog gave rise to two groups. One evolved into a pre-vertebralis adapted for aquatic conditions, probably shallow water at high altitude where the umbraculum later evolved as a protection against UV radiation. The other group evolved into a smaller, terrestrial species which lived in grassland and moved into the forests which were present at high altitude. These two groups served as the stock from which all the present species in this study evolved – they have been separated for a long time, showing consistent differences in larval and skeletal morphology. The smaller semi-forest form will be referred to as pre-hymenopus.

Pre-vertebralis gave rise to a slightly smaller, more terrestrial species during a period when climatic conditions permitted expansion to the south. Speciation culminated in a pre-fuscigula which expanded into the Cape and flourished due to its large size, relative to the other anurans in the area, and ability to colonize the available marshy habitat. Pre-vertebralis had developed a combined mating and territorial call, which was simply the mating sequence vocalized after the clicking territorial sequence. Pre-fuscigula developed its mating call out of the territorial call of pre-vertebralis, placing emphasis on the first phase of the call, which became much longer than that of pre-vertebralis as a result. Speciation was probably accelerated when the emphasis of the call changed, as reproductive isolation was ensured – pre-fuscigula were attracted by the same call which repelled pre-vertebralis. Pre-fuscigula

retained the mating call second phase of pre-vertebralis, developing a close-range direction finding signal from it. The more terrestrial nature of pre-fuscigula was manifest in a reduction of webbing and a shift of the blood-oxygen dissociation curve to the right of the ancestral pre-vertebralis curve. Climatic conditions were probably unfavourable for the northward expansion of this form, so that pre-fuscigula lived at the Cape, while pre-vertebralis was found in the grasslands on top of the high mountains.

Fringe populations of pre-fuscigula eventually gave rise to a still smaller form, adapted to the drier savanna conditions to the north. This pre-angolensis possessed an increased colonizing ability which enabled it to spread into the African savanna. Its webbing was further reduced, but the blood-oxygen dissociation curve remained adapted to semi-terrestrial savanna conditions. Pre-angolensis was probably widespread, even colonizing the high mountains. The onset of moister conditions lead to the development of forest in Natal, which effectively isolated pre-fuscigula at the Cape, a population of pre-angolensis on the Drakensberg, and the bulk of pre-angolensis in areas to the north. During this phase pre-angolensis had developed a mating call which placed more emphasis on the second phase, which tended to have an FM component. The call was also abbreviated. Once the spread of forest had isolated the Drakensberg pre-angolensis from the rest of the population, the development of a high-altitude left-shifted dissociation curve was able to occur. Presumably the pre-angolensis was a more adaptable form, as the population of pre-vertebralis never developed a left shifted curve to such a degree. This Drakensberg population of pre-angolensis stabilized as form "D".

Meanwhile the pre-angolensis populations of the northern savanna evolved into an even more successful colonizing species, with a range of morphs and a sophisticated mating call. The mating call of this northern form was a continuation of the development seen in form "D": the second phase was emphasized and refined. This resulted in a reduction in the length of the first phase, and an increase in the length of the second phase. This species stabilized as angolensis, and began to expand as the climate became drier and the forest retreated.

The final picture is of four distinct species: vertebralis and "D" on the Drakensberg, fuscigula at the Cape and angolensis to the north. Angolensis and fuscigula now contact each other over a broad zone of sympatry.



Meanwhile pre-hymenopus gave rise to fringe populations which were adapted to forest conditions. These speciated into a form of pre-wageri by raising the frequency of the mating call, probably developing the territorial frequency into the new mating call frequency. The morphology and mating call structure of pre-hymenopus and pre-wageri remained similar. Pre-wageri adapted to the forest environment by evolving simple colour morphs, and attaching eggs to submerged vegetation.

Either pre-hymenopus or pre-wageri later gave rise to a semi-savanna form which split into two species, pre-fasciata and pre-grayi. Pre-fasciata adapted to the grassy vlei environment which was unoccupied by either pre-fuscigula or pre-angolensis. This radiation may have started in clearings in the forest, accelerating as the forest was replaced by grassland during drier conditions. Pre-grayi expanded into the remaining disturbed and shaded environments not utilized by any of the other species. Pre-grayi retained the forest breeding habit of terrestrial oviposition, and was successful in this vacant niche. Both pre-grayi and pre-fasciata shared a simple single-pulsed mating call, which was a contraction of the ancestral "pulse train" mating call possessed by pre-wageri and pre-hymenopus. Pre-fasciata developed colour morphs for camouflage in dry grass, while pre-grayi evolved a range of colour morphs, predominantly with browns, reflecting its preference for areas where the vegetation was brown or shaded. The protective colouration of the morphs, and the terrestrial breeding enabled pre-grayi to thrive in habitats which were marginal for the other species. The forest origin of these two species can be seen in pre-fasciata's habit of climbing, and pre-grayi's terrestrial oviposition. The forest adapted pre-hymenopus has therefore given rise to four species : hymenopus, wageri, fasciata and grayi.

As the forest retreated, wageri was left in relict populations while grayi and fasciata were able to expand north and south. At the present time hymenopus is restricted to the rocky slopes of the Drakensberg escarpment, where it is meeting competition from grayi. Wageri is disappearing with the few remaining patches of forest, but is surviving on the lower wooded areas of the Drakensberg. Fasciata is very successfully occupying the grassy vleis of the savanna, although in wetter areas it occurs with grayi, angolensis and fuscigula. Grayi is utilizing habitats which are unsuitable for other members of the group. In very

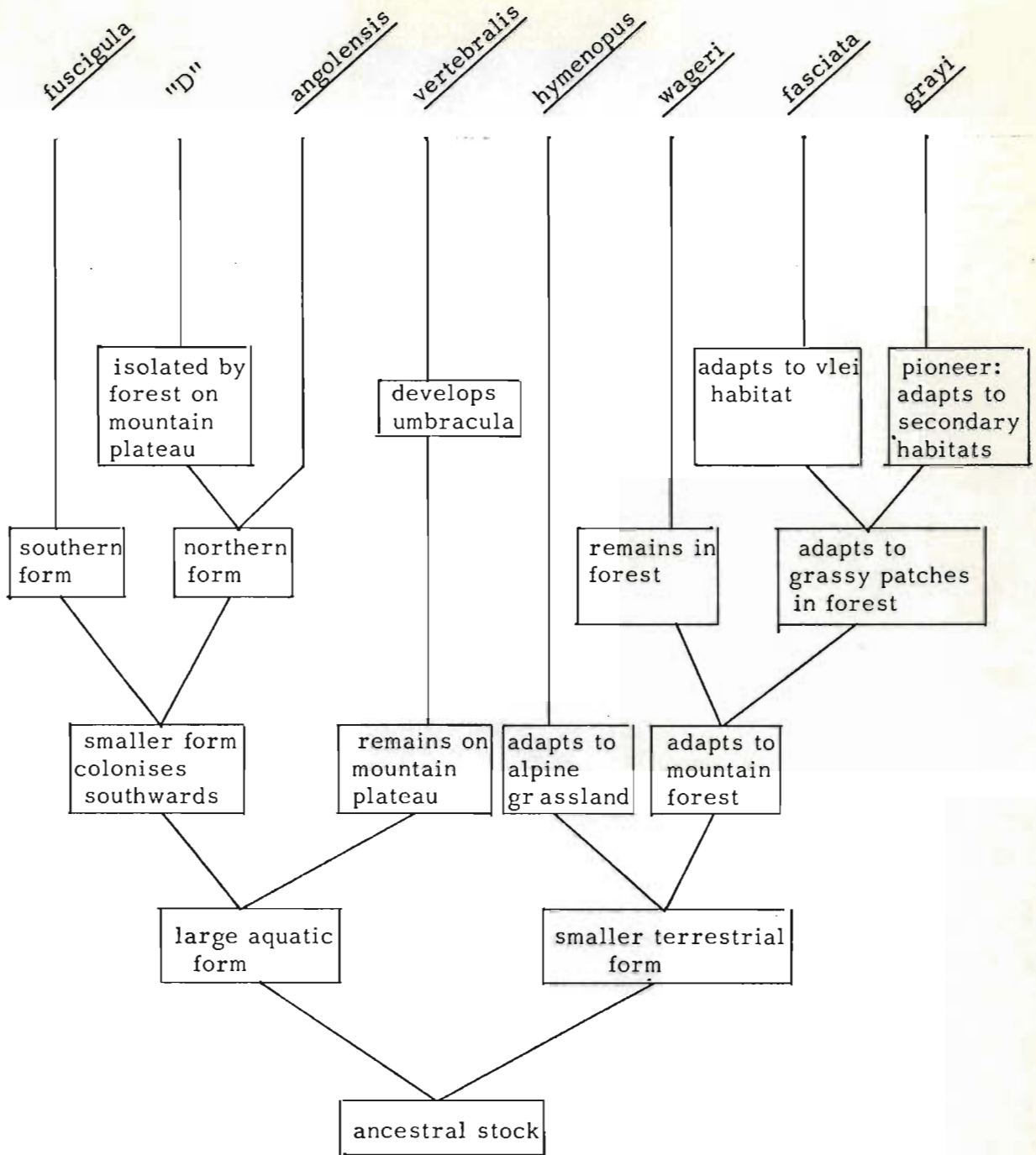


Figure 47b. Summary of the proposed evolutionary sequence.

recent times it has been able to occupy a large number of secondary habitats created by man, like ditches, gardens and agricultural lands.

As both grayi and angolensis are widespread successful species with many morphs, future speciation would be expected to arise in their populations. Subspecies of grayi and fasciata are currently recognised (Inger 1959, Poynton 1964a). Ecological and behavioural studies are required of these subspecies to determine if and how speciation is occurring and what the selection pressures might be.

## 9.2 Systematic implications of this study

The eight species studied fall naturally into two groups which are adaptively different. On the one hand is a group of four species of large frogs with a probable savanna origin, and on the other hand a group of four species of smaller frogs with a probable forest origin. I propose to place the smaller frogs in the genus Strongylopus Tschudi 1838, after Van Dijk 1966. The two genera, Rana and Strongylopus, can be compared in figure 48. (Data from this study and other southern African studies only.)

	<u>Rana</u>	<u>Strongylopus</u>
Species included	<u>R. angolensis</u> , <u>R. fuscigula</u> , <u>R. vertebralis</u> , <u>R. sp.</u> (form D)	<u>S. hymenopus</u> , <u>S. wageri</u> , <u>S. fasciata</u> , <u>S. grayi</u>
Maximum size after Poynton (1964a)	65mm-140mm	46mm-65mm
Pectoral girdle	not notched (omosternum)	notched (Poynton omosternum 1964a)
Tadpoles	Genera consistently different in larval morphology, especially mouthparts (Van Dijk 1966)	
P <sub>50</sub> of blood	1,7kPa-2,7kPa	3,1kPa-6,3kPa
Most common webbing	0-2 phalanges of fourth toe free	3-4 phalanges of fourth toe free
Mating call	biphasic	single phase

Figure 48. Comparison of the genera Rana and Strongylopus in southern Africa. Data from this study unless otherwise acknowledged.

The adaptive differences displayed by the two genera support Inger's (1958) definition of genera. When based on detailed studies, splitting of cosmopolitan genera like Rana can only increase our understanding of the group as a whole. Rana and Strongylopus are closely related and compliment each other ecologically. When considering the species in each genus, it appears that the most recent species have been selected for a eurytopic, colonizing way of life.

### 9.3 Key to the two genera

I suggest that the following couplet be used after arriving at "Rana" in any existing key to the genera of southern African frogs:

1. 0-2 phalanges of fourth toe free of webbing, omosternum not notched. Mating call consists of two phases; an initial "clicking" sequence followed by a "buzz". Large frogs up to 140mm from snout to urostyle.

RANA

- 2 3-4 phalanges of fourth toe free of webbing, omosternum notched. Mating call consists of a single note or train of pulses. Small frogs rarely exceeding 65mm from snout to urostyle.

STRONGYLOPUS

Van Dijk's (1966) tadpole key recognizes both genera.

### Note on the Karyotype of Strongylopus

Strongylopus grayi possesses a karyotype of  $2n=26$ , which is the same as the complement for typical Rana (Scheel J.J., 1971. Hereditas 67: 287-290).



## 10 HELEOPHRYNE NATALENSIS

This little known species belongs to the only genus of the family Leptodactylidae present in southern Africa. Its closest relatives are apparently the Leptodactylidae of South America and Australia. At the commencement of this project the distribution records in the Natal Museum from the study area were St. Heliers, Kloof, Pietermaritzburg and Cathedral Peak. The ecological interaction of this species with Rana and Strongylopus was unknown, but all three genera were known to occur along streams.

### 10.1 Distribution

The localities at which Heleophryne natalensis was found in the study area are shown in figure 49. The tadpoles of this species proved useful in locating populations. They possess large oral suckers with which they cling to rocks in swift flowing streams, while they feed by rasping algae off rocks, leaving distinctive "graze trails". These graze trails were found to be excellent markers for Heleophryne natalensis as they could be clearly seen during the day when the adults and tadpoles were concealed. The presence of tadpoles was easily established in areas where graze trails were found, by turning over a few stones until specimens were located.

### 10.2 Habitat selection

The localities at which Heleophryne was recorded varied in altitude between 320-2300m, with mean slopes (see 7.2) of 4° and more.

Tadpoles are always found in fast flowing rocky streams, frequently near or on waterfalls. The tadpoles are well adapted for clinging on to the rocky substrate with well developed oral suckers, and are found on sheets of rock over which water is flowing rapidly, or actively climbing up vertical rock faces. When disturbed the tadpoles release their hold of the rock and are washed downstream out of danger. During the day the tadpoles are concealed under rocks or in dark, shaded pools. At night they emerge to feed on the algae which grows on rocks in the water.

The adults remain concealed under rocks and in cracks during the day, emerging after dark to feed in the vegetation along the streams, and on rock ledges associated with waterfalls. Fast flowing streams with waterfalls are usually found with forest and natural bush in the study area, and this has come to be regarded as typical Heleophryne habitat. However, on a recent collecting trip in the Cathedral Peak Forest Reserve, Mr. Orty Bourquin collected a Heleophryne adult from a hole in a mud bank. The



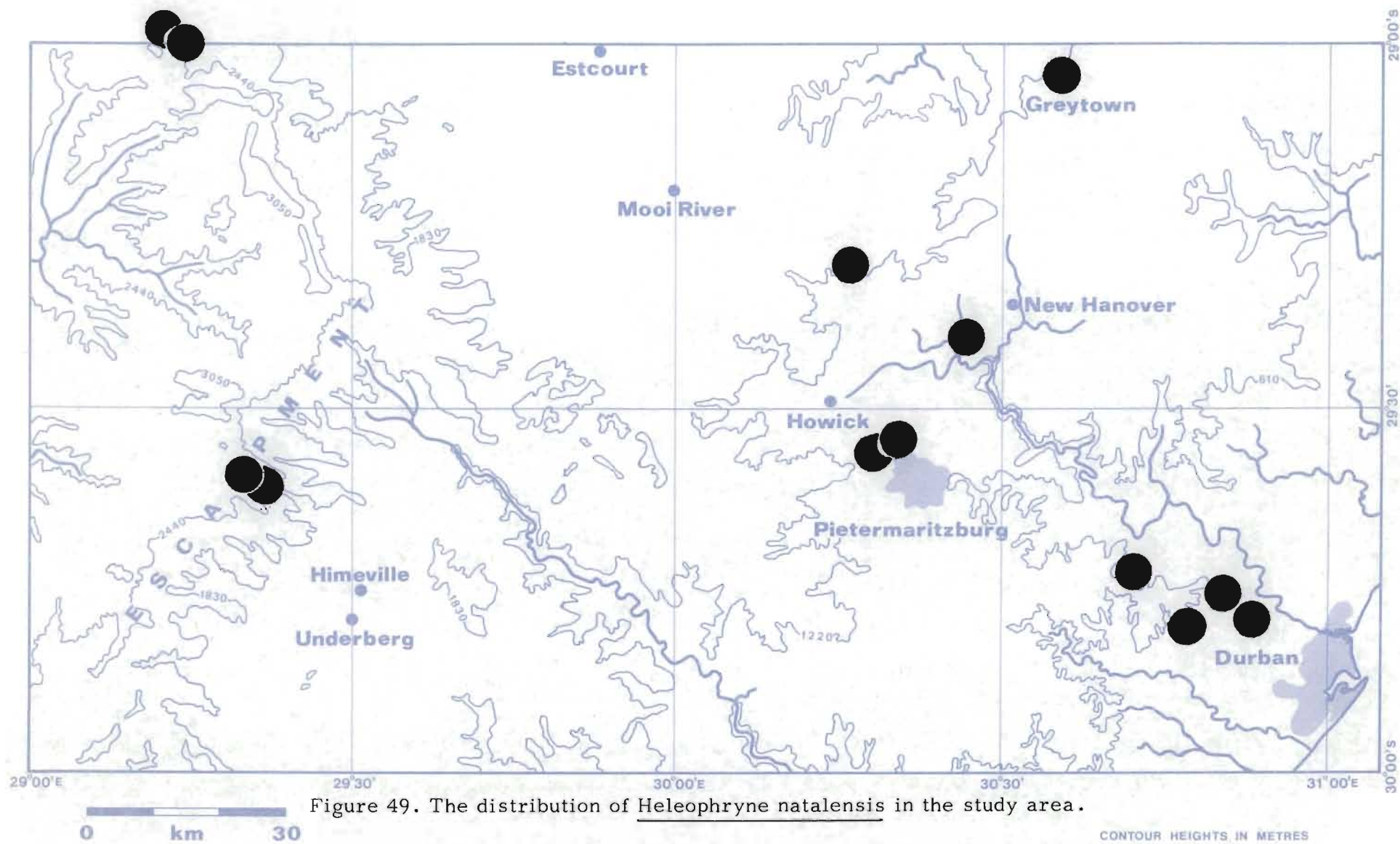


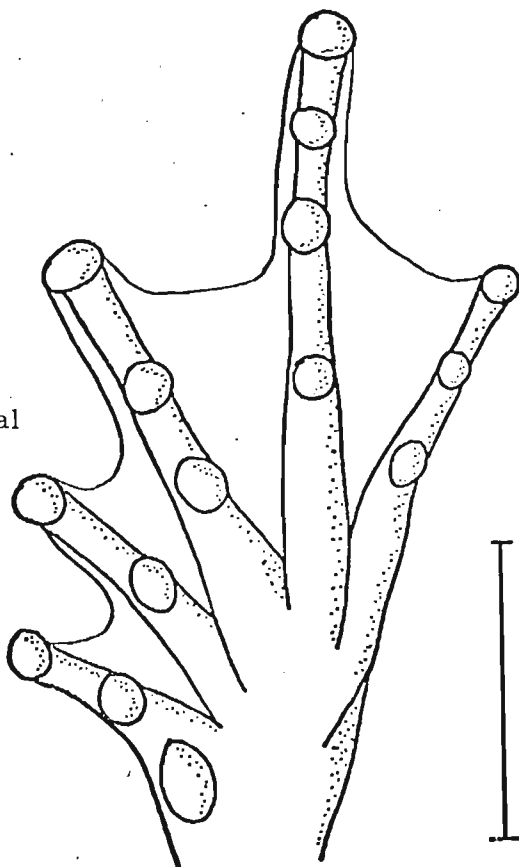
Figure 49. The distribution of *Heleophryne natalensis* in the study area.

mud bank is situated on a spur under the Gewaagd beacon at 2225m , in open grassland many hundreds of metres from the nearest stream. On another occasion while we were climbing in the same area, we reached a pit which the forestry department had dug for soil tests. The pit was just over a metre deep, situated on a grassy watershed at 2317m. Mr. Bourquin jumped into the pit and found a Heleophryne, despite the doubts expressed previously by other members of the party. These two records indicate that Heleophryne adults are not confined to shaded mountain streams, even though they always breed in such situations.

### 10.3 Adaptations to life on waterfalls

Heleophryne natalensis possesses well developed plantar and digital tubercles, and a thick web between the toes (figure 50) with which it is able to climb around on rock ledges near waterfalls. Dorsoventral flattening enables adults to creep into narrow crevices and under rocks. Studies on the blood of this species , using the methods described previously (2.1) reveal the following (n=9): mean haematocrit 26,8% (range 19-35); mean haemoglobin concentration 7,2g/100ml (range 5,6-8,6); mean erythrocyte count  $5,24 \times 10^5$  per  $\text{mm}^3$  (range  $3,55-6,80 \times 10^5$ ).

Figure 50. Drawing of the left foot (ventral surface) of a typical Heleophryne natalensis to show the well developed tubercles and webbing. Scale is 10mm.



The blood oxygen dissociation curve (figure 51) shows that the  $P_{50}$  of Heleophryne blood is 1,8 kPa. This species is adapted to climb and creep into crevices, and is typically found submerged or in damp spots under rocks or in cracks. Linear transforms of the equilibrium curve show that the slope  $(n) = 0,94$ . This compares closely with known hyperbolic dissociation curves.

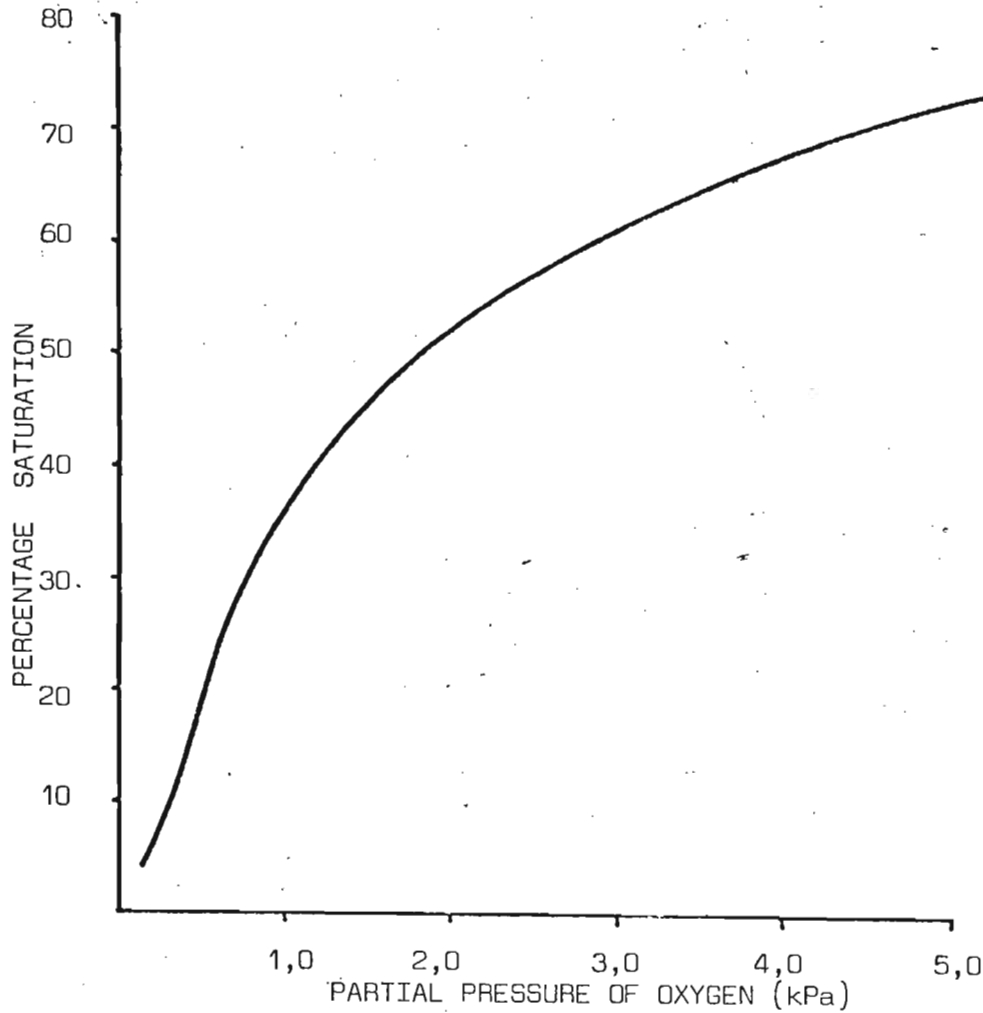


Figure 51. Dissociation curve of the blood of Heleophryne natalensis.

#### 10.4 Competition with Rana and Strongylopus

Comparison of the blood values of Heleophryne with fig. 6 show that Heleophryne is similar to Rana and Strongylopus in terms of haematocrit, erythrocyte count and haemoglobin concentration. The  $P_{50}$  of Heleophryne is much lower than the species of Strongylopus, indicating an ability to take up oxygen from an aquatic environment. Heleophryne has the most left-shifted curve of all the species studied, apart from Rana sp. (form "D"), suggesting an aquatic ancestry.

Tadpoles of Heleophryne are restricted to rocky, fast flowing streams. Although tadpoles of R. angolensis and S. hymenopus have been found in the same streams as Heleophryne, they do not occupy the same area - Heleophryne lives in the rapidly flowing sections, while Rana and Strongylopus tadpoles are found in deep pools or quiet backwaters. Heleophryne tadpoles feed by rasping algae off rocks, while Rana and Strongylopus tadpoles are detritus feeders. It appears that the tadpoles of Heleophryne are not in competition with tadpoles of Rana or Strongylopus for space or food.

The adults sometimes feed in the same areas along the edges of streams. Heleophryne's ability to climb enables it to avoid the foraging areas of Rana and Strongylopus, although competition for food probably does occur at ground level. Extensive field studies of the general biology of Heleophryne natalensis are required.

In conclusion, it can be said that H. natalensis is a climbing frog, adapted to live in areas of steep slope and rapid streams, commonly found near waterfalls where it avoids competition from R. angolensis, S. grayi and S. hymenopus. The species is not common, but can be found in small numbers at sites in natural bush. These sites typically occur in gullies or against cliffs where there is a perennial stream.



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FITZPATRICK, R.W. Periglacial soils with permafrost horizons in southern Africa. (Proc. 4th S. Afr. Soc. Quat. Res. 1977)  
Ann. Natal Mus.

## APPENDIX

## TECHNIQUE FOR OXYGEN EQUILIBRIUM CURVE DETERMINATION

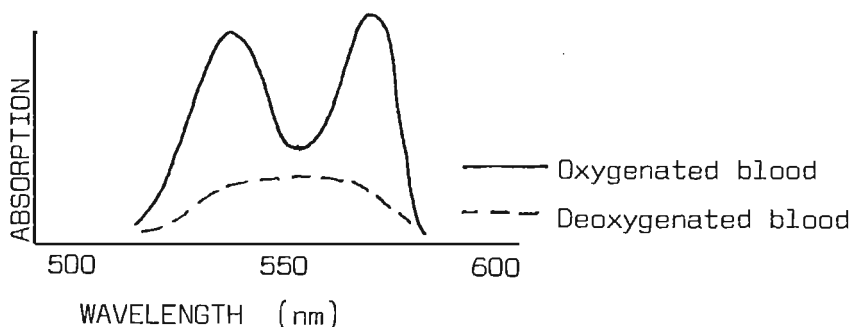
1. Preparation of 100% and 0% saturation samples

All equilibrations were carried out in the micro-tonometers which form part of the Radiometer BMS 2 "Astrup" equipment. They are basically small glass test-tubes, attached to a shaker, with a side arm connected to the gas supply. About 50 microlitres of blood is placed in a tonometer, which is then shaken while gas is passed over the blood surface. The tonometer is immersed in a waterbath, and the gas supply is saturated with water vapour in a water trap before passing into the tonometer. The gas is regulated to a flow rate of between 400–500 ml/minute.

To prepare the 100% saturated standard, a cylinder of pure oxygen was connected to the gas supply. Equilibration was carried out for 15 minutes, as longer contact with oxygen results in denaturation of the blood. A 0% saturated standard is prepared by equilibrating the blood with pure nitrogen for 40 minutes.

2. Determination of the two wavelengths at which the sample is to be measured and construction of a calibration curve.

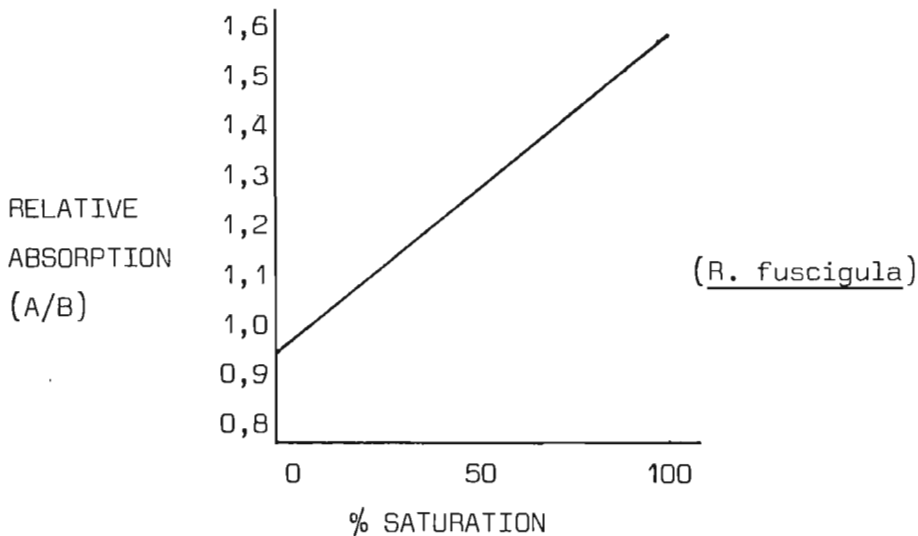
After equilibration, the oxygenated sample is drawn up into a micro-haematocrit tube which contains about five microlitres of Triton X borate solution (33ml Triton X-100: 67ml 0,1M borate). The sample is then stirred using a magnet to move a small piece of wire which is inserted in the tube. The Triton X solution is a detergent which haemolyses the blood. The sample is then led into a short-path-length cuvette (0,1mm), and scanned on a Beckman 25 spectrophotometer. The 0% saturated sample is treated in the same way. The figure below shows how the absorption at different wavelengths varies between oxygenated and deoxygenated blood.





The wavelengths of the peak (A) and the trough (B) are determined on the plotted scan and then checked on the spectrophotometer. The relative increase in absorption at wavelength (A), expressed as absorption at (A)/absorption at (B), is proportional to the percentage saturation of the blood. The calibration curve for this ratio is linear, as shown by the following figure:

CALIBRATION CURVE OF BLOOD SATURATION/SPECTROPHOTOMETRIC ABSORPTION



The wavelengths (A) and (B) were similar for all nine species examined. The table below lists the wavelengths and the relative absorptions of the standard solutions for each species:

SPECIES	PEAK(A)	TROUGH(B)	0% A/B	100% A/B
<u>R. angolensis</u>	577nm	560nm	0,84	1,66
<u>R. sp. (D)</u>	577nm	560nm	0,84	1,66
<u>R. fuscigula</u>	577nm	560nm	0,94	1,53
<u>R. vertebralis</u>	578nm	562nm	0,94	1,63
<u>S. grayi</u>	578nm	560nm	0,85	1,53
<u>S. fasciata</u>	578nm	561nm	0,85	1,52
<u>S. wageri</u>	578nm	563nm	0,92	1,49
<u>S. hyemenopus</u>	578nm	560nm	0,88	1,65
<u>H. natalensis</u>	578nm	562nm	0,91	1,81

### 3. Equilibration of blood samples at various partial pressures of oxygen.

I used the following gas mixtures (percentage oxygen in nitrogen): 0,6%; 1,2%; 2,8%; 6,6%; 8,8%. Each blood sample was equilibrated for at least 30 minutes. To shorten the duration of each set of equilibrations, each sample was pre-equilibrated at the next lower oxygen pressure for 15 minutes (while a second sample was equilibrating at the same pressure), and then equilibrated at the final pressure for 15 minutes (while a new sample was pre-equilibrated at the same pressure). This was possible because the BMS 2 apparatus used has a pair of tonometers which can be linked to the same gas supply. No difference in saturation was found between this process and the more lengthy procedure of equilibrating each sample for 30 minutes at each partial pressure. Staggering the use of the tonometers in this fashion permitted almost double the number of determinations to be processed, as one sample could be measured while another was equilibrating.

### 4. Measuring the sample absorption, and determining the percentage saturation.

The spectrophotometer used was a Beckman 25, which was set to "micro" with normal slit width. The absorption of the sample was read at the two wavelengths selected in step 2, and a ratio of reading A/reading B determined. This ratio could be read off the calibration curve to determine the percentage saturation of the sample. In practice I found that the use of a hand calculator which was pre-programmed produced accurate results without the need to draw the calibration curve. The following equation was used: percentage saturation =  $(100\% \text{ standard ratio} - \text{sample ratio})100 / 100\% \text{ standard ratio} - 0\% \text{ standard ratio}$ .

## POLYACRYLAMIDE GEL ELECTROPHORESIS

The following running and stacking gels were used:

### Stock solution A

48 ml 1N HCl  
36,3 g Tris base  
0,46 ml TEMED (N,N,N',N'-tetramethylethylenediamine)  
H<sub>2</sub>O to 100 ml, pH 8,9

Stock solution B

6,4 ml 1M phosphoric acid  
 1,45 g Tris base  
 water to 100 ml, pH 6,9

Stock solution C

45 g Acrylamide  
 1,2 g MBA (N,N'-methylenebisacrylamide)  
 water to 100 ml

Stock solution D

10 g Acrylamide  
 2,5 g MBA  
 water to 100 ml.

AP

14 mg ammonium persulphate in  
 10ml water (made fresh every  
 time)

Reservoir buffer

12 g Tris base  
 57,6 g Glycine  
 water to 1 litre, pH 8,3 (diluted 1:10 to use)

Parts by volume (ml) for running and stacking gels

Solution	A	B	C	D	AP	H <sub>2</sub> O
Running gel	1	-	1,3	-	4	1,7
Stacking gel	-	1	-	1	1	1 (15 microlitres TEMED added last)

The application buffer used was a solution containing 0,01 M beta-mercapto-ethanol, 10% glycerol, and 1/5 diluted solution B.

## ENZYME IDENTIFICATION

The following stains were modified from Cooksey (1971) (Cooksey, K.E. Disc Electrophoresis. Chap. 10. Meth. Microbiol. 5B: 573-594):

Esterase stain (made up fresh immediately before use)

20mg 1-naphthylacetate in 2ml acetone (substrate), 20mg Fast Blue B (dye), 100ml 0,01M phosphate buffer pH 7,2. Gels are soaked in this solution in the dark for five to fifteen minutes, until the bands appear where enzyme activity is present.

Phosphatase stain (made up fresh immediately before use)

20mg B-naphthyl phosphate (substrate), 20mg Fast Blue B (dye), 100ml  
0,01M Tris/HCl buffer pH 8,3.

Lactate dehydrogenase stain (made up fresh immediately before use)

2ml saturated sodium lactate solution (substrate), 30mg NAD, 1,4mg  
phenazine methosulphate, 80mg Nitro-Blue tetrazolium (dye), 100ml  
0,01M Tris/HCl buffer pH 8,3

Both the above stains were used in the dark for five to fifteen minutes at room temperature until the bands appeared.

## INTERPRETATION OF SONAGRAMS

The terminology describing sonagrams is not yet stabilized. The reader may wish to refer to the explanations below when examining figures 25 and 26, and their discussions.

Amplitude is represented by intensity of shading. Darker shades indicate higher amplitudes.

Amplitude modulated (FM) sounds show regular on-off-on-off characteristics frequently with distinct gaps in the trace along the time axis.

Dominant harmonic. The harmonic which carries the most energy (also called the emphasized frequency). It is recognisable by its high amplitude relative to other harmonics when these are present.

Frequency is represented on the Y axis, and measured in kHz.

Frequency modulated (FM) sounds may be recognised by the variations in vertical position of the sonagram trace.

Fundamental frequency (pitch) is the frequency at which the vocal apparatus is vibrating. It can be determined by measuring the frequency difference between two successive harmonics. The fundamental frequency is not always present on sonagrams, as animal sounds are often complex, with portions of the call emphasized and others damped.

Harmonic. A simple multiple of the fundamental frequency, represented on a sonagram by a horizontal band. A series of harmonics may be present, but certain harmonics may be damped.

Phase. I have regarded each structurally uniform part of a complex vocalization as a phase. Thus the call of R. angolensis consists of two phases, an



initial clicking phase and a terminal phase.

Pulse. Each pulse is represented on a sonagram by a fine vertical line.

Pulse train. A sound consisting of a number of discrete pulses. Two types are recognized in these sonagrams:

- 1) Complex pulse trains, where the pulses are grouped into units, and the units are grouped into pulse trains (S. hymenopus, fig. 26).
- 2) Simple pulse trains, where the pulses are not grouped into units but are separated by regular intervals (R. fuscigula, fig. 25).

Unit. A number of closely spaced pulses, which are separated by a longer interval from the next group of pulses.