Aust. J. Biol. Sci., 1975, **28**, 89–108

Chromosomal Evolution in the Lizard Genus *Varanus* (Reptilia)

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Abstract

The karyotypes have been determined of 16 of the 32 species of the genus *Varanus*, including animals from Africa, Israel, Malaya and Australia. A constant chromosome number of $2n = 40$ was observed. The karyotype is divided into eight pairs of large chromosomes and 12 pairs of microchromosomes. A series of chromosomal rearrangements have become established in both size groups of the karyotype and are restricted to centromere shifts, probably caused by pericentric inversion. Species could be placed in one of six distinct karyotype groups which are differentiated by these rearrangements and whose grouping does not always correspond with the current taxonomy. An unusual sex chromosome system of the *ZZ/ZW* type was present in a number of the species examined.

The evolutionary significance of these chromosomal rearrangements, their origin and their mode of establishment are discussed and related to the current taxonomic groupings. The most likely phylogenetic model based on chromosome morphology, fossil evidence and the current distribution of the genus *Varanus* is presented.

Introduction

The lizard genus *Varanus* is an ancient group of platynotan reptiles with uncertain evolutionary affinities (Bellairs 1969). Members of the genus are commonly known as the monitors or goannas and are found in a circum–Indian–Ocean distribution ranging throughout Australia, S.E. Asia, India, Pakistan, the Middle East, the U.S.S.R. and Africa. The greatest density and diversity of species occurs in Australia and S.E. Asia, with 28 of the 32 species and 7 of the 10 subgenera being present in this region.

The varanids are of particular interest as it is believed that they have comparatively recently extended their range from S.E. Asia westward into Africa (Keast 1971). The main evidence for this viewpoint is based on the distribution and diversification in the eastern part of their range, the absence of ancient fossil evidence from Africa and Madagascar and the total absence of varanids from Madagascar (Blanc 1972).

Current chromosomal data on this genus are very limited; Matthey (1949), Dutt (1969), Gorman and Gress (1970*b*) and Singh *et al.* (1970) have determined the karyotypes of only four species. Unfortunately, although showing a constant chromosome number of $2n = 40$, the karyotypes they presented lacked accurate centromere placement and were of poor resolution. The question as to whether the varanids possess a sex chromosome system and if so which sex was heterogametic also remained unanswered. This is an area of considerable evolutionary interest, as a number of quite different sex chromosome systems have already been reported in lizards (see White 1973 for review).

This report presents a detailed chromosomal analysis of the genus *Varanus* with emphasis on certain aspects of its evolution. Specifically, the aims were:

1. To investigate the type, mode of origin and the direction taken by chromosomal rearrangements.
2. To determine whether a sex chromosome system has become established in the group and to examine its probable evolutionary origin.
3. To construct a phylogeny based on chromosomal morphology and to relate this to current ideas on varanid radiations and evolution.
4. To investigate the taxonomic implications of these evolutionary changes.

Methods

Short-term leucocyte cultures were prepared with 0·15 ml of whole blood per culture. The blood was extracted by heart puncture or from the ventral tail vein using a sterile heparinized syringe. The culture was made up with 4·0 ml of medium 199 [Commonwealth Serum Laboratories (C.S.L.)] or Eagles M.E.M. (C.S.L.), and a growth supplement of 1 ml of calf serum or foetal calf serum (C.S.L.) was added. Cultures were stimulated with 0·05 ml of phytohemagglutinin M (Difco). Cell growth in these cultures was often very poor and media preference was highly erratic. Consequently the chromosome observations are limited to gross morphology and 'banding' techniques proved technically impossible.

The cultures were grown at 32°C for 4 days and 0·02 ml of 0·02% *N*-desacetyl-*N*-methylcolchicine (Colcemid; Ciba) solution was added 4–6 h before harvesting. The procedures for harvesting and production of slides are described by Gorman *et al.* (1967). Slides were stained with 10% R66 Giemsa solution (Gurr) in phosphate buffer (pH 7) for 3 min.

The chromosome number was ascertained for each species by counts on all well-spread mitotic metaphase cells. Chromosome measurements were made on cut-out photographic karyotypes of at least five well-spread metaphase cells per species at a magnification of × 3000. The mean percentage total chromosome length of each chromosome arm, the arm ratios and their standard errors were calculated.

The species examined, their origin and the number of specimens studied in each case are listed in Table 1. Where possible, specimens have been lodged in the collections of the South Australian Museum or the Australian Museum (N.S.W.). A number of live specimens are still retained in private zoological collections (see Acknowledgments).

Results

A constant chromosome number ($2n = 40$) was observed in all specimens of the species examined in this study. This is consistent with the number found in *V. gouldii* (Matthey 1949), *V. monitor* (probably *V. bengalensis*) (Dutt 1969, *V. rudicollis* (Gorman and Gress 1970*b*) and *V. flavescens* (Singh *et al.* 1970). The karyotype of varanids is characterized by eight pairs of large chromosomes and 12 pairs of microchromosomes. The former are subdivided into two pairs of large metacentric elements (pair 1 having a conspicuous secondary constriction on the short arm) and six pairs of chromosomes of a slightly smaller size and with varying centromere positions.

As the karyotypes of numbers of species had no significant measurement differences between them, these species are described in their karyotype groups rather than in their conventional taxonomic listing.

Group A; Subgenus Varanus (*Fig. 1*)

This group included the following species: *V. gouldii gouldii, V. gouldii rosenbergi, V. gouldii flavirufus, V. giganteus, V. mertensi* and *V. spenceri.* These Australian species have the characteristic varanid karyotype described above, with chromosome

pairs 3, 4 and 8 being metacentric and pairs 5, 6 and 7 clearly acrocentric. The microchromosomes (pairs 9–20) are also acrocentric. There was an absence of any chromosomal heteromorphism, and there were no significant size differences between the karyotypes of these species.

Table 1. Localities, numbers and sex of specimens examined

The subgeneric groupings given are based on the classification of Mertens (1963). Numbers in parentheses are the number of species in each subgenus

Taxonomic grouping of species	No. of specimens ♀	?	♂	Locality
Subgenus *Odatria* (13)				
V. gilleni (Lucas & Frost)		1		Renmark, S.A.
V. storri (Mertens)			1	Queensland
V. semiremex (Peters)		3		Edward R., Qld
V. tristis orientalis (Fry)	1		1	Queensland
V. timorensis similis (Mertens)			2	Darwin, N.T.
Subgenus *Varanus* (10)				
V. varius (Shaw)	2		2	Armidale, N.S.W.; Renmark, S.A.
V. indicus indicus (Daudin)		3		Edward R., Qld
V. salvator salvator (Laurenti)	1	2	2	Singapore
V. gouldii gouldii (Gray)			5	Renmark, S.A.; Cape York, Qld; Western Australia
V. gouldii flavirufus (Mertens)			3	Lake Eyre, S.A.
V. gouldii rosenbergi (Mertens)			3	Kangaroo I., S.A.; Western Australia
V. giganteus (Gray)			1	Northern Territory
V. spenceri (Lucas & Frost)			1	Queensland
V. mertensi (Glauert)		1	1	Darwin, N.T.; Canon Hill, N.T.
Subgenus *Indovaranus* (1)				
V. bengalensis nebulosus (Gray)			1	Singapore
Subgenus *Psammosaurus* (1)				
V. griseus griseus (Daudin)			2	Israel
Subgenus *Empagusia* (2)				
V. exanthematicus albigularis (Daudin)	1			Umtali, S. Rhodesia
Subgenus *Polydaedalus* (1)				
V. niloticus niloticus (Linnaeus)	1			Umtali, S. Rhodesia

Group B; Subgenus Varanus (*Figs 2 and 3*)

The two species in this group, *V. varius* and *V. indicus indicus*, differ from the previous group in that pairs 3, 4, 6, 7 and 8 are metacentric, whilst pair 5 is distinctly acrocentric. Chromosome pairs 9–20, the microchromosomes, have varying centromere positions, many of them being clearly metacentric. However, the possibility of establishing the exact number of metacentric microchromosomes was restricted by their size and resulting problems with resolution.

The largest pair of microchromosomes in *V. varius* (pair 9) was clearly heteromorphic. In the female an acrocentric chromosome was paired with a metacentric one of half its size, whilst in the male there were two small metacentric chromosomes. Although only two specimens of each sex were examined, this would appear to be a sex chromosome system of the *ZZ/ZW* type, i.e. female heterogamety (Figs 2 and 3).

The three specimens of *V. indicus indicus* that were examined did not show this heteromorphism. However, the sex of the specimens could not be determined, since

they could not be killed and dissected and the possibility of heterogamety cannot be excluded at this stage.

Group C; Subgenera Varanus *and* Indovaranus (*Figs 4 and 5*)

The S.E. Asian species *V. salvator salvator* and *V. bengalensis nebulosus* belong to this group. Their karyotypes are very similar to those in group B, except that the short arm of the acrocentric chromosome pair 5 appears to be markedly longer, though this difference is not statistically significant (see Table 3). It was also noted that all microchromosomes were distinctly acrocentric. There were no significant measurement differences between the karyotypes within this group. Chromosomal heteromorphism was not observed in any specimens examined, although in some cases the sex could not be ascertained.

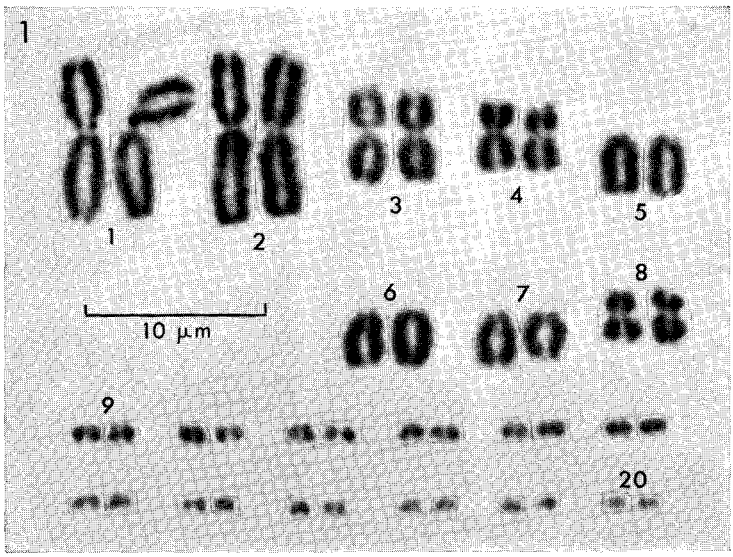


Fig. 1. Karyotype of the group A species, *V. gouldii gouldii* (male).

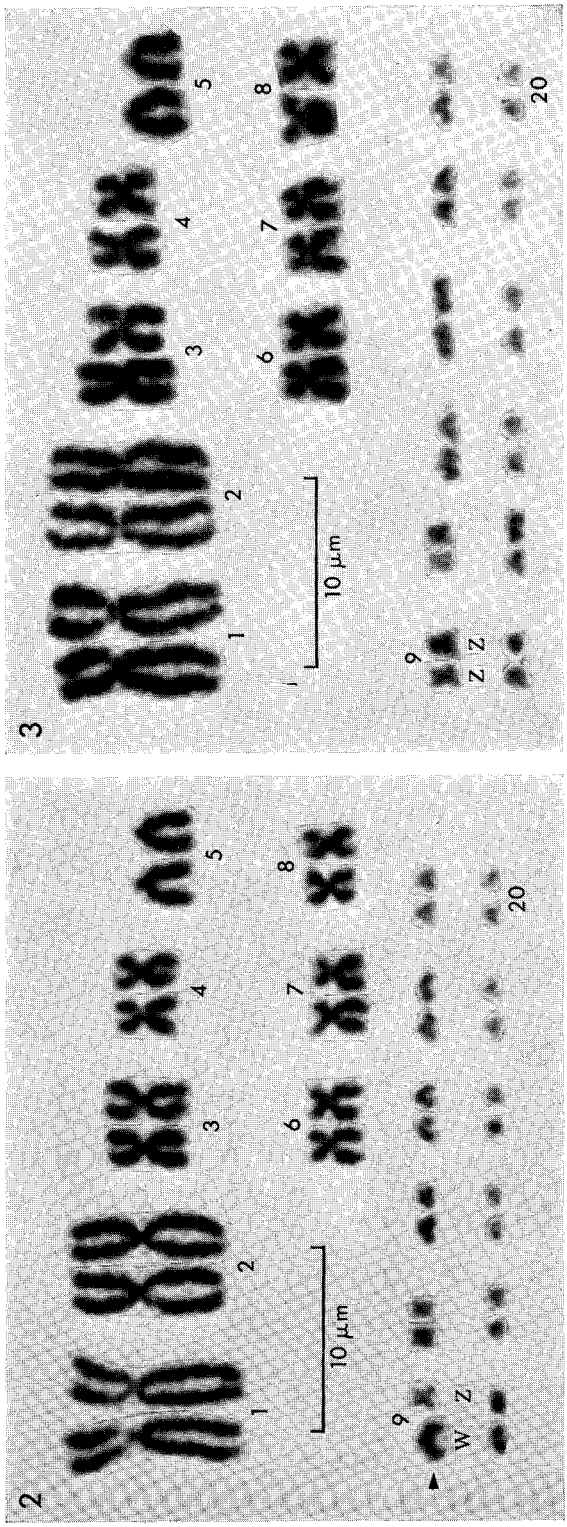
Group D; Subgenus Odatria (*Figs 6–8*)

The species *V. semiremex*, *V. gilleni*, *V. timorensis similis*, *V. tristis orientalis* and *V. storri* were assigned to this group. Their karyotypes are very similar to those of groups B and C: the elements numbered 3, 4, 6, 7 and 8 are metacentric whilst pair 5 is acrocentric and of the group B type. The microchromosomes of most species examined were acrocentric.

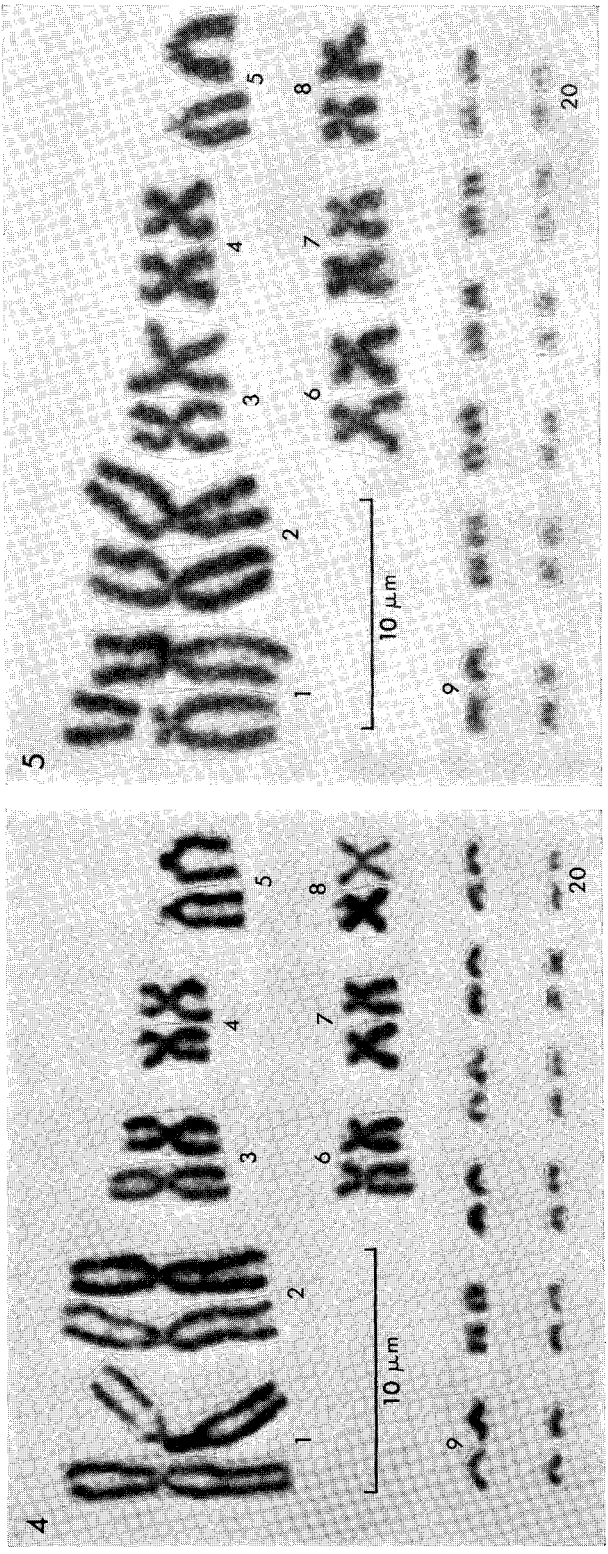
A number of cases of inter- and intraspecific variability was observed. In *V. storri* (Fig. 7) the microchromosomes of pair 10 were conspicuously metacentrie, whilst they were acrocentric in the other species of this group. Moreover, an individual of *V. tristis orientalis* (Fig. 8) was found to have a secondary constriction on the long arm of pair 7 which was not observed in another specimen of the same species.

Group E; Subgenus Psammosaurus (*Fig. 9*)

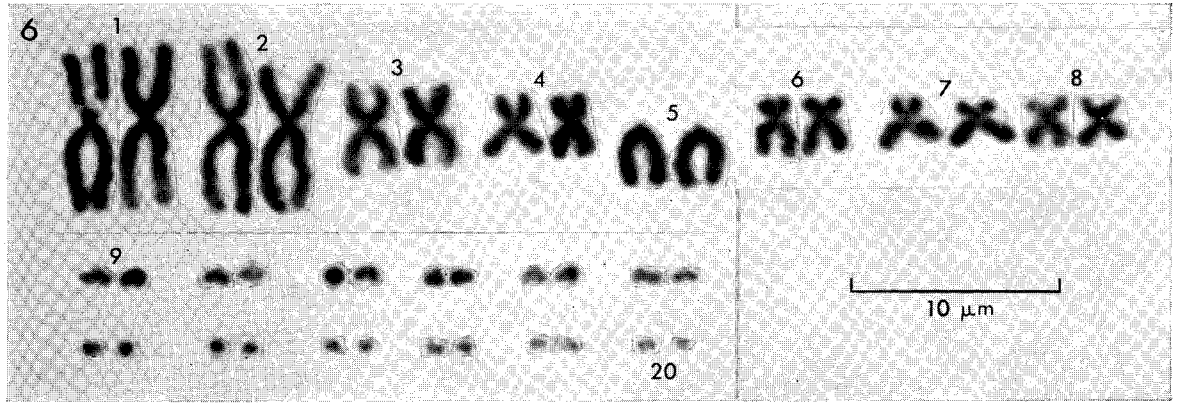
A single Middle Eastern species, *V. griseus griseus*, had chromosomes that differed from all others examined in that pairs 5, 6, 7 and 8 were distinctly subacrocentric.

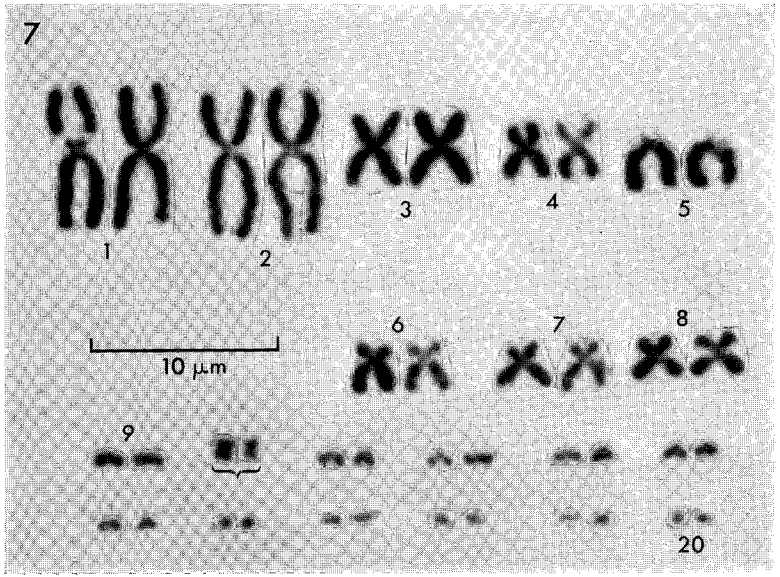


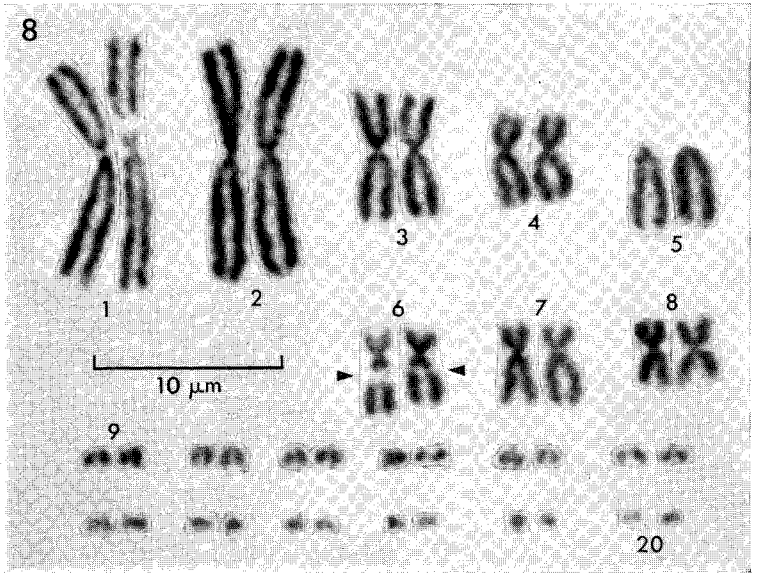
Figs 2 and 3. Karyotypes of female (Fig. 2) and male (Fig. 3) *V. varius* (group B). Note the heteromorphic pair of chromosomes in the female (arrow).



Figs 4 and 5. Karyotypes of the group C species, male *V. bengalensis nebulosus* (Fig. 4) and male *V. salvator salvator* (Fig. 5).







Figs 6-8. Karyotypes of representatives of group D.
Fig. 6, *V. gilleni* (sex unknown).
Fig. 7, Male *V. storri*.
Fig. 8, Male *V. tristis orientalis*.
Note the secondary constriction in pair 6 of *V. tristis orientalis* (arrows) and the two metacentric microchromosomes (pair 10) in *V. storri* (brace).

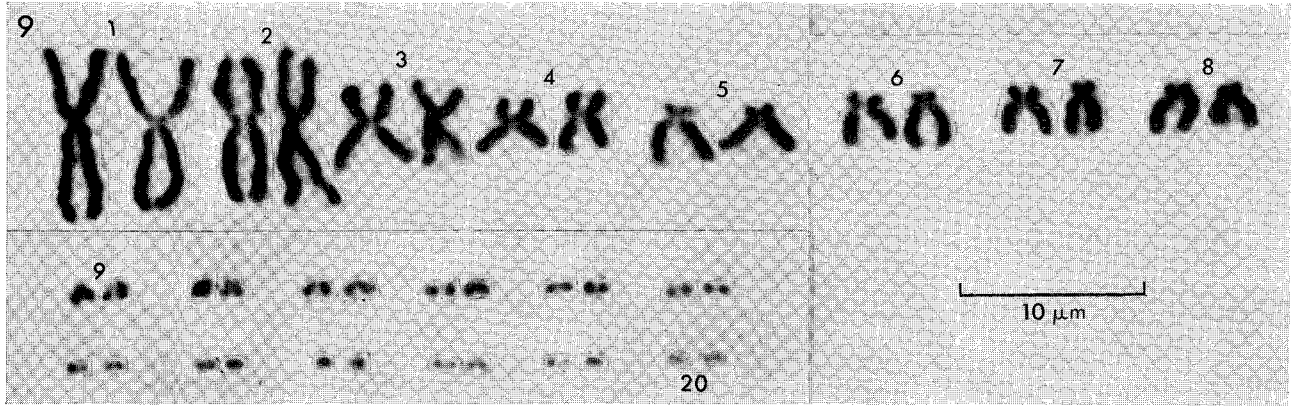
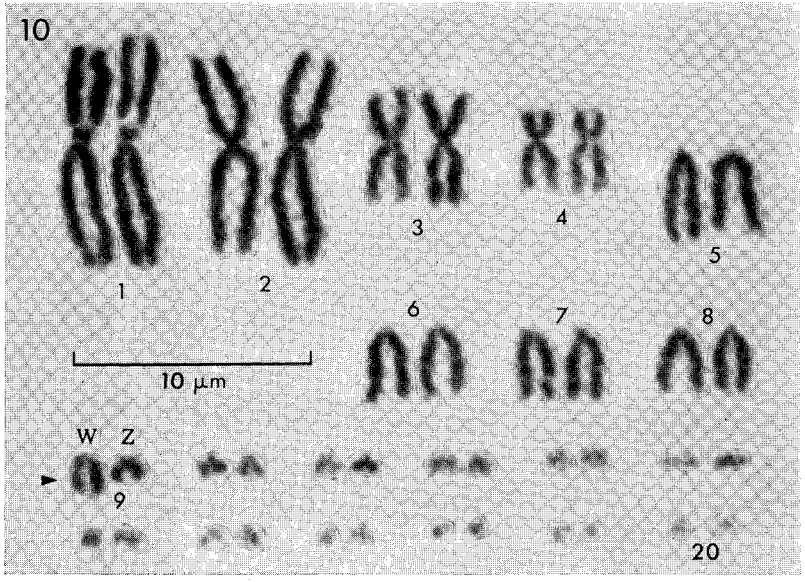
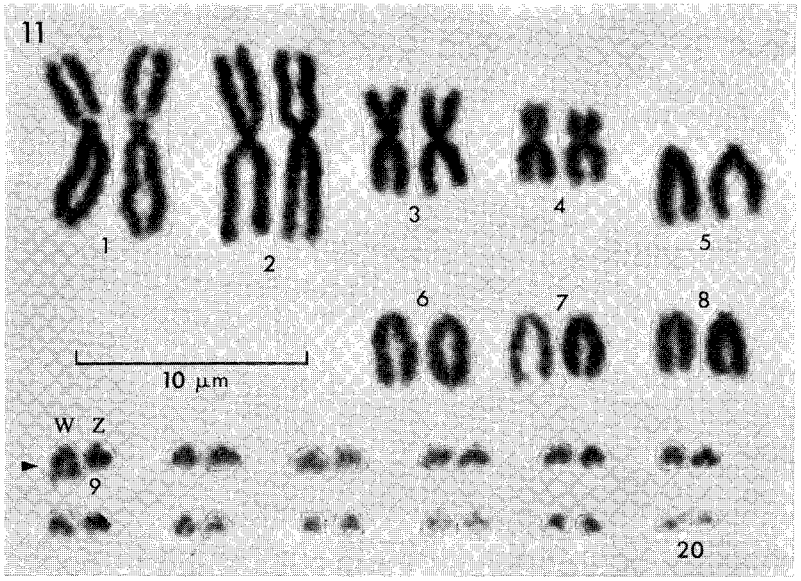


Fig. 9. Karyotype of male *V. griseus griseus* (group E).

Figs 10 and 11. Karyotypes of the group F species, female *V. exanthematicus albigularis* (Fig. 10) and female *V. niloticus niloticus* (Fig. 11). Both species have a heteromorphic pair of micro-chromosomes (pair 9; see arrows).





The microchromosome pairs 9–20 were observed to be mainly metacentric. The single specimen analysed lacked any apparent chromosomal heteromorphism.

Group F; Subgenera Empagusia *and* Polydaedalus (*Figs 10 and 11*)

Mitotic chromosomes of the two species in this group (*V. exanthematicus albigularis* and *V. niloticus niloticus*) were not significantly different from each other in any measurements taken. However, they were markedly different from all other karyotype groups. Chromosome pairs 3 and 4 were metacentric whereas pairs 5, 6, 7 and 8 were acrocentric. Moreover, the microchromosomes (9–20) were in many cases metacentric. An obvious heteromorphism similar in appearance to that of *V. varius* was observed in the microchromosomes of pair 9. As only single female specimens of each species were available, the precise nature of this mechanism could not be determined, though it would appear to be a *ZZ/ZW* sex chromosome system of the *V. varius* type.

A number of general trends have been observed in the chromosomal evolution of this genus. Firstly, karyotypic rearrangements have been restricted to centromeric shifts probably caused by pericentric inversions. These inversions have occurred in both the pair 5–8 size group and the microchromosomes (pairs 9–20). Species within most karyotypic groups have shown conservation of chromosome morphology; however, species in group D have both inter- and intraspecific chromosome variability.

A sex chromosome system of the *ZZ/ZW* type (female heterogamety) is present in a number of the species examined. However, the difficulty of obtaining specimens and of sexing specimens belonging to private collections has prevented an assessment of the universality of this mechanism.

A summary of the chromosomal variation found between species and groups is presented in Table 2. The geographic distribution of these karyotype morphologies is shown in Fig. 12. It should be noted that the arm ratios and percentage total chromosome length values presented in Table 3 are for representative species from each karyotypic group, since there were no significant differences between species within groups.

Unfortunately, it was not possible for us to obtain blood from the remaining species in the subgenus *Varanus* (*V. komodoensis*, *V. karlschmidti* and *V. mitchelli*), eight rarer species of the subgenus *Odatria* and the three monospecific subgenera *Tectovaranus*, *Philippinosaurus* and *Papusaurus*. However, chromosomal data are available for *V. rudicollis* (Gorman and Gress 1970*b*) and *V. flavescens* (Singh *et al.* 1970). Although only the former report has an illustration of the karyotype, the latter including a description only, it would appear that both of these species possess the *V. salvator* karyotype morphology (group C).

Discussion

(*a*) *Chromosomal Evolution*

Detailed chromosomal analyses have been made on only a very few groups of lizards and snakes which are mainly North American in origin. These studies have shown that the predominant form of chromosomal rearrangement in lizards is centric fusion [Cole (1970) in the genus *Sceloporus* (Iguanidae), Lowe *et al.* (1970) in the genus *Cnemidophorus* (Teiidae), Bury *et al.* (1969) in Anguidae and Huang and Gans (1971) in Amphisbaenidae]. It was also noted that pericentric inversion has played

a significant but less pronounced role in the evolution of these taxa and that in some instances centric 'fission' has occurred (Huang *et al.* 1967; Webster *et al.* 1972).

Table 2. Characteristics of the karyotypic groups studied

Group[A]	Species	Female with heteromorphic pair 9 (*ZW*)	No. of acrocentrics in pairs 5–8	No. of sub-acrocentrics in pairs 5–8	Acrocentric micro-chromosomes	Metacentric micro-chromosomes
A	Subgenus *Varanus*					
	V. gouldii gouldii	?	3	0	+	–
	V. gouldii rosenbergi	?	3	0	+	–
	V. gouldii flavirufus	?	3	0	+	–
	V. giganteus	?	3	0	+	–
	V. spenceri	?	3	0	+	–
	V. mertensi	?	3	0	+	–
B	Subgenus *Varanus*					
	V. varius	+	1	0	–	+
	V. indicus indicus	?	1	0	–	+
C	Subgenus *Dendrovaranus*					
	V. rudicollis[B]	?	0	1?	?	?
	Subgenus *Empagusia*					
	V. flavescens[C]	?	0	1?	?	?
	Subgenus *Indovaranus*					
	V. bengalensis bengalensis[C]	?	0	1	?	?
	V. bengalensis nebulosus	?	0	1	+	–
	Subgenus *Varanus*					
	V. salvator salvator	–	0	1	+	–
D	Subgenus *Odatria*					
	V. gilleni	?	1	0	+	–
	V. storri	?	1	0	–	+(1)
	V. tristis orientalis	–	1	0	+	–
	V. semiremex	?	1	0	+	–
	V. timorensis similis	?	1	0	+	–
E	Subgenus *Psammosaurus*					
	V. griseus griseus	?	0	4	–	+
F	Subgenus *Empagusia*					
	V. exanthematicus albigularis	+	4	0	–	+
	Subgenus *Polydaedalus*					
	V. niloticus niloticus	+	4	0	–	+

[A] Grouped according to karyotype. [B] Gorman and Gress (1970*b*). [C] Singh *et al.* (1970).

The data presented in this study indicate that all chromosomal differences except for the pair 9 heteromorphism (see Discussion, section *b*) can be accounted for by pericentric inversion. The rearrangements have been restricted to two areas in the karyotype, i.e. pairs 5–8 and the microchromosomes. The remaining larger elements have retained a consistent morphology throughout the evolution of this taxa.

Chromosome pairs 5–8 have been a particularly active area for karyotypic evolution, as at least 11 pericentric inversions have been established in the surviving species. Presumably, many more of these rearrangements in other chromosomes

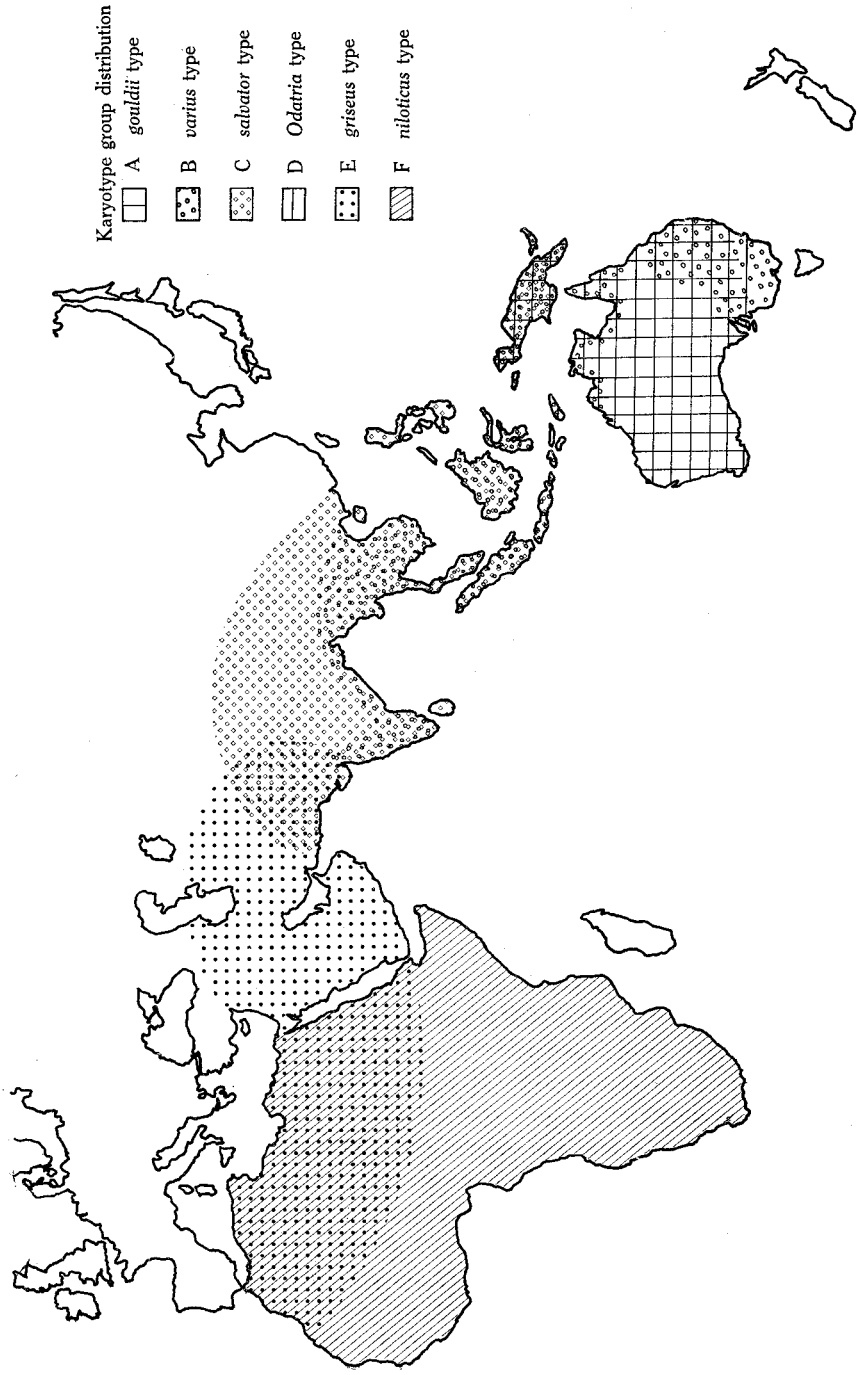


Fig. 12. The world-wide distribution of each of the karyotypic groups.

never reached fixation, having occurred in structurally unsuccessful forms, or were present in now extinct species. A possible explanation for this high incidence of successful pericentric inversions is that these particular chromosomes may have a series of gene blocks prone to environmental selective pressures. The positioning of these gene blocks could provide the basis for the karyotypic orthoselection (White 1973) of one of the inversion forms. Such a model could account for the apparent convergence of pairs 5–8 in both the Australian *gouldii* group and the African *niloticus* group. Holmes, King and King (unpublished data) have also found evidence of convergence in a series of conservative enzyme systems detected by gel electrophoresis, which parallels the karyotypic situation, i.e. these Australian and African forms are electrophoretically more alike than is either to any other. All available evidence shows that this is due to convergence rather than being a relic of an ancient affinity i.e. continental drift (see Discussion, section *c* i).

Table 3. Percentage total chromosome length (%TCL) of chromosome pairs and their arm ratios (AR) ± standard errors for representative species of each karyotype group

Chromosome pair	Group A *V. gouldii gouldii*	Group B *V. varius*	Group C *V. salvator*	Group D *V. storri*	Group E *V. griseus*	Group F *V. niloticus*
1 %TCL	19·69	19·53	18·94	17·88	19·49	18·54
AR	1·20±0·03	1·30±0·05	1·24±0·04	1·18±0·06	1·21±0·07	1·17±0·03
2 %TCL	17·73	17·76	18·22	17·40	17·82	18·29
AR	1·26±0·02	1·18±0·02	1·23±0·05	1·26±0·02	1·17±0·06	1·17±0·03
3 %TCL	10·13	9·62	10·39	9·93	10·64	10·32
AR	1·14±0·03	1·11±0·02	1·16±0·04	1·03±0·02	1·19±0·04	1·10±0·02
4 %TCL	7·52	7·22	8·12	7·68	7·69	7·51
AR	1·13±0·02	1·15±0·03	1·08±0·04	1·14±0·06	1·31±0·06	1·27±0·06
5 %TCL	7·09	7·50	8·20	7·11	7·95	7·61
AR	54·8±6·13	9·79±0·88	6·33±1·56	11·77±2·84	2·27±0·15	25·61±4·50
6 %TCL	6·64	6·46	7·42	6·96	6·82	6·85
AR	85·67±10·23	1·49±0·03	1·48±0·05	1·48±0·03	2·20±0·23	30·50±7·27
7 %TCL	6·26	6·10	6·97	6·72	6·67	6·30
AR	34·71	1·40±0·05	1·28±0·06	1·50±0·07	3·17±0·18	18·00±5·36
8 %TCL	6·14	5·92	6·56	6·03	5·51	5·90
AR	1·22±0·04	1·11±0·03	1·32±0·08	1·35±0·17	2·58±0·17	25·20±10·75
9 %TCL	2·23	2·70	1·77	2·32	2·05	2·00
20 %TCL	1·36	1·32	0·98	1·30	1·28	1·24

A possible mechanical explanation for this karyotypic convergence is simply that the attainment of telocentricity in a system evolving by pericentric inversion precludes a return to metacentricity. This is based on the assumption that a break is a relatively rare event and has an equal chance of occurring at any point on a chromosome arm except the centromere. Therefore, the chance of two breaks occurring, one on either side of the centromere to produce a pericentric inversion, would be reduced if one of the chromosome arms involved became conspicuously shorter. Such a situation would result in the inability to form a pericentric inversion if that chromosome attained telocentricity. On this basis, those chromosomes of the 5–8 size group in the African *niloticus* and Australian *gouldii* groups that are telocentric have reached the end point of chromosomal evolution by pericentric inversion. Resolution precludes an accurate assessment of the number of acrocentric or telocentric chromosomes or both in this case.

This explanation does not apply to the microchromosomes. In these elements chromosomal changes appear to have been from acrocentricity to metacentricity. This is particularly noticeable in *V. storri* in the subgenus *Odatria*, where one pair of

the microchromosomes is metacentric, whereas in other species of this subgenus the microchromosomes are acrocentric. Although they have undergone fewer recognizable changes than the larger elements, the microchromosomes have retained their acrocentric morphology in some karyotype groups (A, C and D). Varying levels of metacentricity have been established in the African karyotype groups (E and F) and the group B Asian species.

Similar changes of microchromosome morphology towards metacentricity have been recorded in other lizards by Lowe *et al.* (1967) in the Iguanidae, Huang *et al.* (1967) in the Amphisbaenidae and Lowe *et al.* (1970) in the Teiidae. Indeed, the majority of lizard species for which there is adequate karyotype data appear to have metacentric microchromosomes. The modification of the internal chromosome structure in this manner may give some mechanical advantage during cell division by providing a more effective form of spindle attachment. However, this may be an oversimplification, as Comings and Mattoccia (1970) have shown differences in heterochromatin and replication patterns in the microchromosomes of *Coturnix japonica* and M. King (unpublished data) has shown similar heterochromatic patterns in the microchromosomes of agamid and scincid lizards.

From these observations it is possible to demonstrate three contrasting lines of chromosome evolution in the varanid lizards, all apparently without change in chromosome number or in the numerical balance between the very small or 'micro'-chromosomes and the larger chromosomes (see Fig. 13). Firstly, there is apparent conservation of the four large metacentric chromosomes. These are presumed to be related in the different species; certainly there is a constant location of an achromatic region, possibly the nucleolar organizer, on one of the largest pairs (see pair 1, all figures). Perhaps banding pattern analyses would provide a test of this presumed relationship. Secondly, there is variation in chromosomes 5, 6, 7 and 8 leading to an accumulation of acrocentric chromosomes in this group. Thirdly, there is an accumulation of metacentric chromosomes in the microchromosomes of three of the cytological groups. The role of the microchromosomes in these and other organisms (e.g. Aves) is not known. Comings and Mattoccia (1970) suggest that they are associated with the nucleolus in the quail, but there is no evidence favouring this role in varanids, and other evidence (the achromatic region present in a large metacentric pair of chromosomes) argues against this. Clearly they represent a special class of chromosome with regard to size. However, they would appear to possess other properties as well, since the formal argument advanced in this paper for the approach to and retention of an extreme acrocentric morphology of some of the large chromosomes is not applicable to these microchromosomes; rather the reverse seems to apply.

The present distribution of species that possess differing karyotype morphologies is of interest not only from a phylogenetic viewpoint but also in terms of the mode of origin of the rearrangements. As can be seen from the distribution map (Fig. 12) all groups overlap in an easterly to westerly direction, the degree of overlap varying considerably. If the phylogenetic model (see Discussion, section *c* ii, and Fig. 13) and the predominant palaeontological and distributional data are accepted, the varanids have undertaken a series of east to west migrations from Asia to Africa. It is possible that inversion differences were established in isolated populations on the periphery of the range of a karyotype morph and probably in a polymorphic state. Homozygosity of this new rearrangement may have been achieved under the intense selective pressures of an adaptive radiation into new territory. The acceptance of the concept

that the changes occurred during the radiations and not independently of them is enhanced by the obvious symmetry in direction of karyotype evolution from the Asian extreme to the African extreme going through an intermediate Middle-Eastern form. Karyotypic changes accompanying radiations have been observed in a number of other organisms, e.g. in *Perognathus goldmani* (Patton 1969) and in North African spalacid rodents (Lay and Nadler 1972).

Except for the subgenus *Odatria*, all other varanid species are characterized by specimens having large size, high mobility, extensive home ranges and wide distribution. These appear to be the ideal criteria for organisms to show pronounced karyotypic stability at the population level (lacking inter- and intrapopulation variation), as has been demonstrated in this study and other studies on organisms with similar characteristics [see Árnason (1972) in whales and seals; Hammar (1970) in birds; Takagi *et al.* (1972) in Ratitae; Baker and Patton (1969) in bats; Taylor *et al.* (1968) in camels].

It is in the *Odatria*, however, that we see species with a very different lifestyle, and a correspondingly different degree of chromosomal variability—i.e. a much higher level of interspecific variation. Most species in this subgenus are very small animals, with restricted home ranges, that occur in isolated populations and have distinct distributions. Other organisms, particularly rodents (Matthey 1963; Patton 1969, 1973; Wahrman *et al.* 1969), flightless *Orthoptera* (White 1973) and gekkonid lizards (M. King, unpublished data) with similar characteristics, also have extreme chromosomal variability.

These data would support the hypothesis that mobility and the degree of genetic isolation that populations of a species can obtain are prime requirements for karyotypic diversity. However, the universality of these criteria are dependent on a multitude of subtle qualities possessed by a particular group of organisms both in terms of chromosome structure and stability and the lifestyle of the species. Clearly the absence of data from a large number of animals of any one species precludes an estimation of the occurrence of intrapopulation variation.

(*b*) *Sex Chromosomes*

Sex chromosome heteromorphism has been reported in a few species in a number of diverse lizard families. Male heterogamety of both the XX/XY and X_1X_1 X_2X_2/X_1X_2Y forms occur in several genera of the Iguanidae [Gorman and Atkins (1966) in *Anolis*, Pennock *et al.* (1969) in *Uta* and Cole *et al.* (1967) in *Sceloporus*]. Species from other lizard families such as the Pygopodidae (Gorman and Gress 1970*a*), the Teiidae (Cole 1969) and the Scincidae (Wright 1973) also possess XX/XY or X_1X_1 X_2X_2/X_1X_2Y sex chromosome systems. In contrast, female heterogamety of the ZZ/ZW type has been observed in the Lacertidae [by Ivanov and Fedorova (1970) in *Lacerta strigata* and by Oguma (1934) and Chevalier (1969) in *L. vivipara*].

Sex chromosome heteromorphism has not been reported in published karyotypic studies of species of *Varanus* (see Gorman and Gress 1970*b*). The present work has shown that a sex chromosome system of the ZZ/ZW type (female heterogamety) is present in *V. varius*. The chromosomes involved are the largest pair of microchromosomes (pair 9). The mechanism is particularly unusual in that the W chromosome is acrocentric and at least twice the size of the small metacentric Z chromosomes. Other varanid species (*V. niloticus niloticus* and *V. exanthematicus albigularis*) were observed to have similar heteromorphic microchromosomes in the female, but the

difficulty of obtaining male specimens has pre-empted the confirmation of a sex chromosome system in these species.

The origin of this particular form of heterogamety is difficult to understand, since it is unlikely that the large *W* chromosome in the heteromorphic pair could have gained its extra length from other elements present. This rules out the possibility of its being formed by a translocation. Other mechanisms postulated for attaining heteromorphism have been by pericentric inversion (Ohno 1966; Cole *et al.* 1969) or by degeneration of the heteromorphic element (Peccinini *et al.* 1971), but these could not be applied. It appears that the large *W* chromosome has been produced by 'chromosome growth', i.e. by additions or duplications of segments onto that particular element. A detailed analysis of this sex chromosome system using Giemsa banding techniques may be of considerable value in finding the type of chromatin involved, and the mechanism of its evolution. Unfortunately, attempts at using these techniques have been unsuccessful.

It is worth noting that, as with other lizard species, only a very few species of *Varanus* possess a heteromorphic sex chromosome system. Only in the advanced forms of vertebrates such as mammals and birds do we observe the trend towards specialized sex chromosome systems becoming the norm. Similarly, it is clear that generalizations as to the type of heterogamety found in reptiles and other lower vertebrates are unwarranted. The array of sex chromosome systems described for the very few lizard species analysed so far supports this contention. It is doubtful if any conclusions as to the reasons for this diversity can be made at this stage. However, it could reflect the outcome of evolutionary experimentation for an effective system, or may be simply an index of the polyphyletic origins of surviving lizard taxa.

(*c*) *Evolution*

(i) *Origins of the Varanidae*

Lizards of the family Varanidae are members of the infraorder Diploglossa and are placed in the superfamily Platynota (= Varanoidea) (Bellairs 1969). Advanced Platynota are found in the Lower Cretaceous of Europe (McDowell and Bogert 1954). During the Cretaceous, aquatic members of the families Aigialosauridae, Dolichosauridae and Mosasauridae were widely spread in Europe, North America, Africa and Asia (Bellairs 1969), and other terrestrial families of the Varanoidea radiated widely but were extinct by the end of the Palaeocene (Hoffstetter 1968).

The oldest known members of the family Varanidae are in the extinct subfamily Saniwanae, from the Upper Cretaceous of eastern Asia and North America (McDowell and Bogert 1954). Unfortunately, knowledge of the Cretaceous fauna of the various continents is too incomplete to enable accurate conclusions to be drawn as to the history of the Varanidae, but Hoffstetter (1968) has recently proposed a scheme for the origins of the Varanidae.

The earliest certain representative of the Varanidae is *Telmasaurus* from the Upper Cretaceous of Mongolia, while the position of other Upper Cretaceous genera which are often assigned to the Varanidae is uncertain (Hoffstetter 1968). The genus *Saniwa*, of the subfamily Saniwanae, from the Palaeocene–Eocene in North America is also known from the Eocene of Europe (McDowell and Bogert 1954), while another varanid family, the Necrosauridae, occupied Europe from the Upper Palaeocene to the Oligocene (Hoffstetter 1968). The oldest known representative of the genus

Varanus, subfamily Varaninae (into which all living varanids are placed), is *V. hofmanni* Roger from the Miocene of eastern Europe (Hoffstetter 1968), although incomplete fragments from earlier strata may belong to this genus. Nevertheless, a large gap exists between the occurrence of members of the Saniwanae and the Varaninae in Europe. The recent discovery of a specimen of the genus *Iberovaranus*, which appears to be descended from the Saniwanae from the Lower Miocene of Spain (Hoffstetter 1968), means that it was present at the same time as *Varanus* was established in France. This would seem to indicate that the Iberian Peninsula acted as a refuge for the Saniwanae, which had been present in Europe since the Eocene, and there gave rise to *Iberovaranus*. The genus *Varanus* then migrated into Europe (Hoffstetter 1968) and did not originate there as had been suggested by Fejervary (1918).

It has been suggested by Hoffstetter (1968) that the genus *Varanus* differentiated from Asian Saniwanae (yet to be discovered) and migrated into Europe during the Burdigalien, eventually leading to the extinction of the descendants of the Saniwanae in Europe during the Miocene. The giant extinct *Megalania*, which may be a subgenus of *Varanus* or a closely related genus, is found in Australian fossil deposits which date back to the Miocene (Hecht, personal communication). In addition, fragments of two vertebrae which belong to the Varanidae have been found in beds from the Lower Miocene of Kenya, although it is not possible to determine whether they belong to *Varanus* or *Iberovaranus*. The only other fossil remains of varanids from Africa are from the Recent (McDowell and Bogert 1954).

It thus appears that the genus *Varanus* originated in Asia during or before the Lower Miocene and radiated outward, reaching Europe, Australia and possibly Africa by the Miocene. Fejervary (1918) and Keast (1971) suggest that the radiation of the Varanidae into Africa has been a comparatively recent event.

(ii) *Phylogeny*

Evidence supporting a model of recent African invasion is based on distribution, diversification and fossil data. The distribution and diversification of present-day varanids is heavily biased towards S.E. Asia and Australia, since 7 of the 10 subgenera and 28 of the 32 species are present in this region. Only three species from three different subgenera have colonized the African mainland and none are found on Madagascar (Blanc 1972). The absence of varanids from Madagascar is of particular interest as Blanc (1972) felt that many lizard species had colonized this island from the African mainland. Jacobson (1909) has shown the capacity of varanids to colonize by sea when he found that the new islets formed after the volcanic eruption on Krakatoa were colonized by *V. salvator* within 12 years of their formation. This evidence may support a model proposing the recent colonization of Africa. Extensive palaeontological surveys have also shown an absence of *Varanus* from Madagascar, and the only fossil records from the African mainland which are definitely *Varanus* have been in comparatively recent strata (McDowell and Bogert 1954).

In attempting to construct a phylogenetic tree for the relationships and evolution of the genus *Varanus* based on chromosomal data, a number of basic assumptions have been made. It has been reasoned that the karyotype which is common to the greatest number of taxa, and that from which the most simple derivative karyotypes can be produced, is the primordial form. Secondly, as this group is dominated by

pericentric inversions, it has been assumed that the direction of change has been from metacentricity to acrocentricity in all large chromosomes (see Discussion, section *a*, for model). The third precept is that less weight has been applied to the phylogenetic significance of the microchromosomes because of their poor resolution (see section *a*) and that changes in these elements have been from acrocentricity to metacentricity.

On all of these grounds the common S.E. Asian *V. salvator* karyomorph is regarded as being the extant primordial type. This would differ from a hypothetical progenitor (now extinct), which had all metacentric large chromosomes and acrocentric microchromosomes, by one pericentric inversion on pair 5 which produced a subacrocentric element.

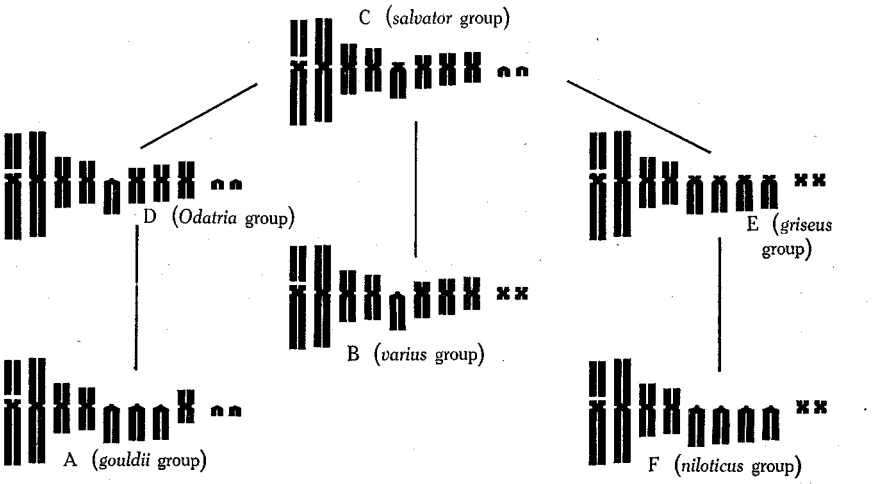


Fig. 13. A diagrammatic representation of each of the haploid karyotype groups, showing their possible phylogeny based on the model outlined in the text. The 12 pairs of microchromosomes are represented by one pair in each group.

The subgenus *Odatria* differs from the *salvator* morph in having one pericentric inversion in pair 5 to produce acrocentricity. The *indicus* group has also established this difference and has had at least two pericentric inversions in the microchromosomes.

The endemic Australian *gouldii* morph differs from *Odatria* by two additional pericentric inversions in pairs 6 and 7 that produced acrocentricity in these elements and by retention of acrocentricity in their microchromosomes.

The *griseus* morph differs from that of *salvator* by an additional three pericentric inversions, thus having four subacrocentric chromosomes, and has established at least two pericentric inversions in the microchromosomes. The *niloticus* group differs from *griseus* by another four pericentric inversions in the same elements, resulting in acrocentricity.

In this model 11 pericentric inversions have been established in the large chromosomes and at least two pairs of inversions fixed in the microchromosomes. The most likely phylogenetic model formed from the above observations and assumptions is presented in Fig. 13 and may have accompanied the following evolutionary trends:

1. The *salvator* form possessing the primordial karyotype ranged throughout S.E. Asia and diversified into a number of distinct subgenera that retained the same karyomorph.

2. There was an initial radiation of varanids into Australia (subgenus *Odatria*) that colonized a series of niches and became specialized as small carnivores.
3. An endemic group (*gouldii*) arose from the *Odatria* in Australia to fill a series of diverse and vacant carnivore niches. It is possible that *Megalania* was an extinct form of this group, being the highest order carnivore that preyed on the giant and extinct macropods.
4. There was a second and very recent invasion of the *indicus* morph from S.E. Asia into northern Australia and down the east coast. The main reason for assuming this to be a recent invasion is the peripheral distribution of species and the absence of *V. varius* from Kangaroo Island, S.A. (isolated from the mainland 8 000–12 000 years ago (Littlejohn and Martin 1965). *V. gouldii rosenbergi* is present on the island and the habitat is remarkably similar to that on the mainland which *V. varius* occupies.
5. A series of earlier westward radiations from the *salvator* stock also occurred, i.e. *griseus* into the Middle East, Pakistan, India, U.S.S.R. and North Africa.
6. There was a final southern radiation of the African *niloticus* type from the *griseus* range throughout Africa.

Careful consideration has been given to a number of other phylogenetic models based on the assumptions outlined earlier. The above model is the simplest and most direct. Fortuitously, it coincides with all known evidence for the evolution of the genus *Varanus* based on anatomy, palaeontological data, distribution and species diversification.

(iii) *Taxonomic considerations*

In a genus of closely related species such as *Varanus*, karyotypic comparisons may be of some taxonomic value. The scarcity of interspecific chromosomal variability, and the presence and consistency of sizeable intergroup chromosomal differences, may provide a basis for scrutinizing the current subgeneric taxonomy.

It is important to note that when very different species have similar karyotypes, there is no validity in lumping them together in the one taxonomic unit, since other characters of higher resolution may provide evidence for a dichotomy. The species *V. niloticus niloticus* (subgenus *Polydaedalus*) and *V. exanthematicus albigularis* (subgenus *Empagusia*) are a case in point, being karyotypically identical but morphologically quite distinct. However, *V. flavescens*, described by Singh *et al.* (1970), is karyotypically quite different from *V. exanthematicus albigularis*, the other species in the subgenus *Empagusia*. In fact, the chromosome morphology of *V. flavescens* is similar to that of the *salvator* group, it occurs within the range of this karyotypic group and it is an isolate from the African range of *V. exanthematicus*. These points suggest that *V. flavescens* may have closer affinities with the Asian subgenera than with the African *Empagusia* and that further taxonomic studies may be worthwhile.

Similarly, in the subgenus *Varanus* a situation exists wherein chromosomal differences may provide a basis for a more rigorous analysis of the existing taxonomy. The three distinct karyotypic forms described in this subgenus (see Figs 1–5; Table 2) are very different from each other morphologically, yet are consistent in all species within that karyomorph grouping. These are the Australian *gouldii* group (four species), the Australasian *indicus* group (two species) and the Asian *salvator* group (one species). The latter is also karyotypically similar to other subgenera (*Indovaranus* and *Dendrovaranus*). It is therefore possible that the subgenus *Varanus* is

actually masking three distinct taxa and the results reported here may indicate that a more thorough examination of meristic characteristics could reveal less obvious taxonomic criteria.

Acknowledgments

The authors wish to thank Dr D. L. Hayman and Dr D. G. MacPhee for their critical reading of the manuscript. During the course of this study we have received the valuable assistance of a large number of people who have provided us with specimens, allowed us to obtain blood samples from specimens in their collections or helped us in this work in a number of other ways. We gratefully acknowledge the following individuals: J. Armstrong, Dr P. Bavestock, J. Bredl (Renmark Reptile Park), D. Broadley, J. Bull, H. Ehmann, C. J. M. Glover, I. R. Goodwins, Dr B. Green, J. Green (Gosford Reptile Park), Professor M. Hecht, B. Humphries, C. Hurford M.H.R., P. King, Professor H. Mendelessohn, Senator L. Murphy, D. Roberts, R. D. Sharrad, M. Tyler, Dr G. Webb, Dr S. Wheeler, J. White, S. Whitehouse, G. Woerle and J. Wombey.

We wish to thank the Royal Society of South Australia for a grant and the South Australian Museum for assistance on collecting trips. M. King was supported by a University of Adelaide Research Grant.

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Manuscript received 9 August 1974