CHROMOSOMAL EVOLUTION AND THE PHYLOGENY OF ELAPID SNAKES

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All the research described in this thesis, except where acknowledged, represents my own original work.

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Chromosome data are presented for over 100 taxa of snakes, 90% of which belong to the family Elapidae. Utilizing a variety of banding and fluorescence techniques in addition to conventional karyotype analysis these data were examined from two distinct approaches: (1) to determine the nature and degree of chromosome variation within the family, and (2) to use these data to construct karyomorph groupings based on shared fixed differences. When homologies can be assuredly demonstrated from G-banding analysis such groupings are assumed to more accurately reflect relationship than do classifications based solely on morphological characters. In Australian elapids this exercise results in the identification of 10 karyomorph groups which do not always agree with the current generic classification. The karyomorph groups are compared extensively with classifications based on morphological characters and with the limited chemotaxonomic data available in the literature. Based on such comparisons an argument can be raised to reassess *Unechis* and *Denisonia* which are clearly composite genera. Similarly at the species level *Pseudonaja nuchalis*, which has been classified as a single species by taxonomists, probably represents a composite taxon. Some genera on the other hand can be shown to exhibit relationships at a tribal level which has not been
inferred from conventional taxonomy.

The data also provide evidence for specific W chromosome differentiation which, while it cannot as yet be sensibly used to provide a phylogenetic overview, is most certainly taxon specific and so provides a useful marker for specific differentiation. The distribution of nucleolar organizer regions provides evidence on the one hand for differences between African and Asian elapids and on the other hand for interspecific differences between certain of the Australian elapids.

Finally some attention has also been paid to intra-specific chromosome variation in elapid snakes and the first clear demonstration of autosomal polymorphism is presented in respect of the small-eyed snake *Crytophis nigrescens*. This variation demonstrates clearly the interconvertability of micro- and macrochromosomes.
CHAPTER 1
1.1 A Taxonomic Overview

1.1.1 Historical Background to "Elapid" Classification

Although this thesis deals with data collected from the elapid snakes it is important for the reader to understand the taxonomic position of these venomous forms so that the implications of the findings reported in this thesis can be put into a broader perspective.

The basic taxonomy of snakes can be summarized in the following manner:

Order: Squamata (lizards and snakes)
  Sub order: Serpentes (snakes)
  Infra order: Scolecophidia (blind snakes)
    examples: Family Typhlopidae
    Family Leptotyphlopidae
  Infra order: Henophidia
    examples: Family Anilidae (false coral snakes)
    Family Uropeltidae (shield tailed and pipe snakes)
    Family Boidae (boas and pythons)
  Infra order: Caenophidia
    examples: Family Colubridae (includes many harmless and rear-fanged forms often divided into families)
    Family Elapidae
    Proteroglyphous (fixed front fangs)
      (cobras, kraits, mambas, Australian venomous snakes, sea snakes)
    Family Viperidae
    Solenoglyphous (movable front fangs)
      (vipers and pit vipers).

This is not intended as a formal classification, but simply a synopsis of the principal groups involved. Table 1A of the appendix when folded out will provide the reader with details of three of the best known classifications that have been proposed. Table 1B will guide the reader through the taxonomic maze discussed below.

The very sparse fossil record of elapids is first known
from the upper Miocene in France and Moroccó (Hoffstetter, 1962). This record, however, has been of little help in determining precise categories of snake taxa due to a lack of critical fossils (Dowling 1959, Hecht, 1969, Marx and Rabb, 1973), which are completely lacking in Australia, the incomplete nature of most of the fossil forms and consequently the difficulty of recognizing primitive and derived character states in them (Dowling and Duellman, 1975, see also Schwaner and Dessaure, 1981).

Storr (1964) points out that the Elapidae, presumably referring to all terrestrial proteroglyphs, give the appearance of an "old declining group". He also comments that, with the exception of *Naja* (true cobras), all the forty or so then recognized genera form distinct geographic groups endemic to particular continents.

The terrestrial elapids are generally considered to have been derived from a colubrid ancestor. The marine proteroglyphs (sea snakes) are assumed either to have been derived directly from terrestrial elapid snakes or else to share a common ancestral stock with them (see Cogger 1975 for summary). Indeed their close affinities were indicated by Underwood (1967), who attributed confamilial status with a single division between all terrestrial (subfamily Elapinae) and marine (subfamily Hydrophinae) forms (see appendix Tables 1A and B). This was subsequently supported by Hardaway and Williams (1976) following an analysis of the costal cartilages of the ribs. Dowling (1967) also placed all proteroglyphs in the family Elapidae but divided the family into four subfamilies: Apisthocalaminae ("stem elapids"), Elapinae
("terrestrial elapids"), Laticaudinae ("recent sea snakes"), and Hydrophinae ("advanced sea snakes"). Doubt was cast on the monophyletic origin of proteroglyphs by the work of Bourgeois (1965) on the African Mole Viper (Atractaspis) and that of McDowell (1968) on the African elapid snake Elaps (= Homorelaps). Both authors attempted to demonstrate that the closest affinities of their respective forms lay with the same group of rear fanged (opisthoglyphous) African colubrids. This would mean that the two separate venom delivery systems of the Viperidae (solenoglyphous), to which Atractaspis then belonged, and the Elapidae (proteroglyphous), to which Elaps then belonged, would have arisen in parallel from a somewhat similar ancestral stock. In the most recent summaries of snake classification (Smith et al. 1977, Harding and Welch, 1980), which rely heavily on the morphological works of McDowell, Atractaspis has been accorded the status of a separate subfamily within the family Colubridae. Elaps, the type genus for the family Elapidae, appears to be well differentiated from all other members of the group in dental and skull characters. In the light of this, McDowell (1968) adopted the generic name Homorelaps used by Boulenger (1896) thus relegating it to the family Colubridae, a transfer which has not been accepted by all workers (Kochva and Wollberg, 1970).

McDowell (1970) regarded the fossorial African Elapsoidea as among the most primitive of existing elapids together with Bungarus, Boulengerina and to a lesser extent Paranaja. Branch (1979) points out that the absence of apical pits and a loreal scale, two characteristics common to all elapids, may
indicate a fossorial ancestor. These characters are in fact shared with aparallactine colubrids of Africa which also show a fossorial mode of life. This has led both McDowell (1970) and Branch (1979) to conclude that the aparallactines most resemble the presumed ancestral stock.

Among the African elapids the mambas (*Dendroaspis*) can be distinguished from all other forms on the basis of the morphology of the maxilla. In his classical work on reptilian osteology Romer (1956) gave the genus separate subfamilial status (*Dendroaspinidae*) on the basis of this character. Dowling (1959) found this to be an unacceptable criterion for subfamilial distinction. It is interesting to observe that Underwood (1967) describes the skull of *Dendroaspis* as differing from all other African elapids in that the palatine bones lack both choanal and maxillary processes. The Australasian elapids also lack the choanal process and with the exception of *Ogmodon* (Fiji) lack the maxillary process as well. McDowell (1970) elaborated upon the distinctiveness of the dentition and palatine kinesis in *Dendroaspis* but did not remove them taxonomically from other African elapids.

More recently several authors have used a variety of chemotaxonomic characters to infer relationships. Saint Girons and Detrait (1980), for example, have analysed *Dendroaspis* venom in terms of percent common antigens, immunodiffusion, and immunoelectrophoretic comparisons against African (*Naja* and *Hemachatus*), Australian (*Austrelaps* and *Pseudechis*) and Asian elapids (*Naja* and *Bungarus*). They found only minor similarities between the venom of *Dendroaspis* and all the other genera examined. The same authors have
considered, and discounted, the fact that similar life styles and prey are likely to require a similarity in toxicity of the venom. Other authors, however, argue that this may well lead to convergent evolution and, for this reason, should not be used in any phylogenetic interpretation. Saint Girons and Detrait argue that, when they exist, antigenic similarities reflect phylogenetic and not functional affinities. In the same study Saint Giron and Detrait (1980) conclude that the Australian elapids do not constitute a homogeneous group and are not fundamentally differentiated from other terrestrial elapids. This is contrary to the morphological data of McDowell, (1957, 1968, 1969, 1970, 1972, 1974 etc.) Underwood (1967), and Smith et al. (1977) and the venom data of Coulter et al. (1980).

An alternative point of view regarding the origin of the proteroglyph condition has been proposed by Savitsky (1978). He argued that the new world coral snakes, composed of the three genera *Micrurus* (50 + sps), *Leptomicrurus* (3 sps.) and *Micruroides* (1 sp.) and making up approx. 30% of the world's terrestrial elapid fauna, represent an independent derivation of the proteroglyphous condition. He suggests that these two genera share an origin with rear fanged Xenodontine colubrids of South America. Duellman (1979) has supported this idea by removing them to a separate family the Micruridae. McDowell (1967, 1969), on the other hand, had earlier placed the micrurines with a group of semifossorial Asian elapids including *Calliophis*, *Parapistacalamus*, *Maticora* and the sea kraits *Laticauda*. McDowell's classification was adopted by Smith et al. (1977) and three tribes within the subfamily
Elapinae were resurrected namely the Elapini = American and North Asian coral snakes, the Maticorini = South Asiatic coral snakes and Laticaudini = sea kraits.

Again turning to the chemotaxonomic data, the immunological study of Cadle and Sarich (1981) is held to refute the classification proposed by Savitsky (1978) and Duellman (1979). Using microcomplement fixation analysis (MC'F) Cadle and Sarich (1981) have demonstrated a closer relationship between the micrurines, the Asian elapids Ophiophagus and Bungarus, the Australian elapid Austrelaps and the sea kraits Laticauda, on the one hand, than between the micrurines and any of the Xenodonteae colubrids or hydrophine sea snakes on the other. They claim their data unequivocally places the micrurines "on a common lineage with Asian and Australian terrestrial elapids and with the sea snakes". However, as it stands their data neither support nor refute the association of micrurines specifically with Laticauda as proposed by McDowell. In a further immunological study by Cadle and Gorman (1981) no strong association was found between Micrurus and Laticauda. Rather four major lineages of elapid genera were identified: (1) Hydrophine Sea Snakes, (2) Laticauda, (3) New World Coral Snakes and (4) one or more lineages including Demansia (Australian), Bungarus and Ophiophagus (Asian) which were close to none of the reference antisera they studied. Moreover, Demansia was clearly distinguishable from all other Australian elapids examined by these authors. They point out that neither their study nor that of Cadle and Sarich (1981) included either old world Coral Snakes or African elapids. Consequently many of the
relationships suggested by McDowell could not be tested nor could the relationships of the Australian elapids exclusive of Demansia be elucidated.

An affinity between the sea snakes and the terrestrial proteroglyphs, has long been suspected. In a series of studies on proteroglyphs McDowell (1967, 1968, 1969a, 1969b, 1970, 1972 and 1974) analysed a suite of morphological characters, including the osteology of the skull, dentition, venom gland musculature and hemipenal structure. Two important points emerge from his analysis. First, the sea kraite Laticauda is held to have originated quite separately to other sea snakes which collectively form the hydrophines. Second, the structure of the palatine bone and associated processes was held to imply a taxonomic dichotomy based on the functional kinesis of these structures among proteroglyphs. McDowell terms these two groups as "palatine errectors" and "palatine draggers". Most terrestrial elapids fall into the category of "palatine errectors" with the notable exception of the Australian elapid snakes which, like the hydrophine sea snakes, are "palatine draggers" (McDowell, 1970). This information has led Smith et al. (1977) to propose a rather controversial classification in which the Australian elapids are transferred from the family Elapidae to the subfamily Oxyuraninae within the Hydrophiidae. Additionally, in the classification of Smith et al. (1977) the laticaudine sea snakes, formerly a subfamily of the Hydrophiidae, are placed in the tribe Laticaudini, subfamily Elapinae, together with North Asiatic and American Coral Snakes. The remaining elapid subfamily, the Bungarinae, then contains two tribes which
include all Asiatic and African terrestrial elapids (see Appendix, Tables 1A and B).

McDowell considers the laticaudine sea snakes no more than a divergent line within the terrestrial elapids while Voris (1969) concluded, in agreement with Smith (1926), that Laticauda is the most primitive of extant sea snakes though sharing a common origin with the more specialized forms (see Cogger, 1975a). Voris (1977) carried out a multivariate analysis of 43 characters and concluded that the

"Laticauda do not stand on a character-by-character basis between the terrestrial elapids and the other sea snakes ... They (the Laticauda) are very distinct from all other sea snakes and either represent an independent evolutionary line or a very early separation from all other sea snakes. They are by far the most primitive sea snakes and possess many elapid character states".

Such views might be interpreted as bringing the assessment of Voris in closer line to McDowell's claim than his earlier work indicated.

Smith (1931) drew attention to the monotypic genus Ephalophis which he considered primitive and perhaps intermediate between the laticaudine and hydrophine sea snakes. McDowell (1969) placed Hydrophis mertoni in the genus Ephalophis and suggested that through it the hydrophines were derived from the Australian elapids of the "Demansia" group and specifically Drepanodontis (= Hemiaspis) and Rhinoplocephalus (McDowell 1967, 1972 and 1974). McDowell also divided the
hydrophine sea snakes into three groups based on scalation, vertebral anatomy, venom gland musculature and skull morphology, namely:

(1) The **Hydrelaps** group including only the distinctive, and in many respects primitive, genus *Hydrelaps*.

(2) The **Aipysurus** group with *Ephalophis* being the most primitive genus in this group but also including *Aipysurus* and the very specialized *Emydocephalus* which feeds exclusively on fish eggs.

(3) The most advanced **Hydrophis** group containing most of the remaining sea snake genera (see McDowell, 1967 and 1970 for list of generic changes).

This differs radically from Smith's (1926) conception of the family **Hydrophidae** which he believed should contain the subfamily **Laticaudinae**, including *Laticauda, Aipysurus* and *Emydocephalus*, and the subfamily **Hydrophinae**, including *Hydrophis* and all other genera.

Voris (1977) is basically in line with the classification of McDowell (1967, 1970) in pointing out the primitive status of **Hydrelaps**, though he places *Ephalophis* with *Hydrelaps* in a separate group instead of including it among the **Aipysurus** group. He also points to *Hydrophis* (= *Distella*, McDowell, 1972) *kingi*, *H. (D.) major*, *Kerilia jerdoni* and *Thalassophis* as
"relatively primitive" and possibly divergent from the main and more recent Hydrophis stock.

Cogger (1975a) reduces McDowell's (1972) genus Disteria substantially by transferring the species schistosa and stokesii to the genera Enhydrinia and Astrotia respectively, a move that has been adopted by all subsequent authors.

In a brief review of the sea snakes, Burger and Natsumo (1974), reassessed the available data and analysed external morphological features. Attention was again drawn to Ephalophis (Hydrophis) mertoni and, because of the location of the heart, lack of the vestigial left lung seen in E. greyi, and dorsal scale differences, mertoni was considered more advanced than E. greyi and a new genus Parahydrophis was nominated to include only P. mertoni. A new subfamily Ephalophiinae was erected to include five genera which the authors divide into three groups: (1) "Hydrelaps group" for Hydrelaps only, (2) "Ephalophis group" for Ephalophis and Parahydrophis, with Ephalophis selected as the type genus of the subfamily, and (3) "Aipysurus group" for Aipysurus and Emydocephalus. This bears an obvious resemblance to the grouping of genera suggested by McDowell except for the splitting of the Aipysurus group of McDowell and the elevation of these three groups to form a subfamilial assemblage. The remaining subfamily in the classification of Burger and Natsumo (1974) is the Hydrophiinae which includes all other hydrophid sea snake genera, though Disteria (McDowell, 1972 and sensu latu Cogger 1975) has been put back into Hydrophis. They also reaffirm the distinction between Laticauda and the hydrophid sea snakes and emphasize it by placing them in separate
families erecting the **Laticaudidae** for the sea kraits and the **Hydrophidae** for all other sea snakes.

Turning to the pertinent chemotaxonomic data, Mao *et al.* (1977) have examined the structural affinities of the transferrins of *Hydrophis*, *Lapemis*, *Pelamis* ("Hydrophis group" of McDowell), *Aipysurus*, *Emydocephalus* (the "Aipysurus group of McDowell"), *Laticauda*, *Naja* and *Bungarus* (the Elapidae of McDowell) using the MC'F technique. Their interpretation of the data agrees well with McDowell’s (1972) classification with two exceptions: (1) the indicies of dissimilarity suggest that the sea snakes, here including the *Laticauda*, have diverged from Asian terrestrial elapids at the familial level though unfortunately Australian genera were not examined, and (2) the two-way reciprocal titrations between *Laticauda* and *Hydrophis* demonstrate closer affinities between the genera than the morphological criteria indicated, though this does not seem to be supported by Cadle and Gorman's MC'F work (1981 in press).

Mao's data is broadly compatible with the immunoelectrophoretic data of Minton and da Costa (1975) who support many of McDowell's associations with the one exception that *Emydocephalus* was found to be more distinct from *Aipysurus* serologically than it is morphologically. Minton and da Costa also suggest that the sea snakes represent "a homogeneous group closely related to the Australian elapids". From their data *Laticauda* showed greater reactivity to the Australian elapid antisera of *Denisonia* (= *Austrelaps*) than did *Lapemis* and *Hydrophis*. The sample species, however, did not allow the authors to test McDowell’s theory of an Asian rather than an
Australian origin of *Laticauda* or determine its relation to the family Elapidae. In a subsequent review of his own work Minton (1980) concluded that the elapid stocks have probably been distinct since the Miocene though the origin and affinities of the family remain unknown. In the same review Minton describes the Hydrophidae as containing two stocks with *Laticauda* being distinct. The venom analysis of Coulter et al. (1980) found a close relationship between Australian elapids and sea snakes, in agreement with Minton and da Costa (1975), but did not find a close relationship between 'exotic' terrestrial elapid venom (here presumably referring to African and Asian elapids) and Australian venoms.

As mentioned above, the more recent MC'F work of Cadle and Gorman (1981, in press) does include a comparison of sea snakes with both Asiatic and Australian elapids and indicates that sea snakes comprise three groups similar to McDowell's classification (i.e. *Hydrophis*, *Aipysurus* and *Laticauda*). Though the question of a single versus a multiple origin cannot be answered by the immunological data, with the exception of *Demansia psammophis*, the Australian forms are close to both *Hydrophis* and *Laticauda*.

The one thing that should be apparent to the reader from the foregoing discussion is the inability of taxonomists to arrive at any unanimity. This stems directly from the difficulty of defining primitive and derived character states. From the confused taxonomic picture five key questions remain to be resolved at the familial level, namely:
(1) Are the continentally endemic groups of terrestrial elapid snakes confamilial (as suggested by Underwood 1967)?

(2) Do the Australian elapids represent a distinct familial group? (This possibility was also forecast by Underwood 1967 in reference to the work of Storr 1964).

(3) Are Australian elapids a monophyletic group?

(4) What is the precise relationship between the latiscudid and hydrophid sea snakes and the Australian elapids?

(5) Is there evidence to demonstrate an independent origin of hydrophid and latiscudid sea snakes?

Before attempting to answer these questions it is necessary to consider the interspecific taxonomic problems within the Australian elapids.

1.1.2 Introduction to the Taxonomic Relationship within the Terrestrial Australian Elapid Snakes

The two zoogeographic works which include reference to the Australian elapid fauna are those of Storr (1964) and Cogger and Heatwole (1980). Both studies point to the high degree of endemism in Australian elapids. Cogger and Heatwole (1980), for example, find that 94% of all Australian elapid species (approx. 25 genera and 63 + species) are endemic with
the remainder being shared with New Guinea. Only one species of Death Adder (*Acanthophis*) extends to the islands beyond New Guinea. Such a high degree of endemism in Australia is exceeded only by the pygopodid lizards.

Storr (1964) has suggested that Australian elapids are derived from early colubrids, most probably originating in Asia and invading the Australian continent at a time when more primitive colubrids were waning and before the modern colubrid radiation (see also Cogger and Heatwole, 1980). As mentioned above, Storr interprets the high degree of continental endemism to imply that the Elapidae are an old, declining, group. The fossil record is so sparse as to provide no information as to the age of the elapid radiation in Australia, though Cogger and Heatwole (1980) suggest "the major adaptive radiation within Australia in such groups as the elapids snakes, diplodactyline geckoes, the endemic family Pygopodidae and major segments of the lizard families Agamidae, Varanidae and Scincidae are almost certainly derived from elements which arrived no later than the mid-Tertiary. These indigenous radiations, however, apparently proceeded with little or no modification by later migrations of the same families until well into the Quarternary, suggesting that Australia's reptile fauna evolved in virtual isolation between at least mid-Tertiary and the beginning of the Quarternary (a period of 30-35 million years) when a new series of migrations from Asia commenced."

The taxonomic interrelations of the Australian elapid snakes have long been a question for debate. Table 1.1 of this chapter gives some indication of the magnitude of the problem and the variety of classifications that can be derived
## Australian Snakes of the Family Elapidae

<table>
<thead>
<tr>
<th>Genus</th>
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<tr>
<td><em>Acanthophis</em> antarcticus</td>
<td><em>Pseudechis australis</em></td>
<td><em>Oxyuranus</em> sallei</td>
<td><em>Carpetoria</em> sp.</td>
<td><em>Suta</em> serventi</td>
<td><em>Denisonia</em></td>
<td><em>Vermicella</em></td>
<td><em>Pseudechis</em></td>
<td><em>Notechis</em></td>
<td><em>Hoplocephalus</em></td>
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<tr>
<td><em>Australaps</em> superba</td>
<td><em>Cacophis</em> harriettae</td>
<td><em>C. kreftii</em></td>
<td><em>C. squamulosus</em></td>
<td><em>Cryptophis</em> nigrescens</td>
<td><em>C. pallidiceps</em></td>
<td><em>Dendamia</em></td>
<td><em>D. olivacea</em></td>
<td><em>D. paenomorphis</em></td>
<td><em>D. torquata</em></td>
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<tr>
<td><em>Denisonia</em> devillii</td>
<td><em>D. fasciata</em></td>
<td><em>D. maculata</em></td>
<td><em>D. punctata</em></td>
<td><em>Dryadila</em> coronata</td>
<td><em>D. coronoides</em></td>
<td><em>D. mastersi</em></td>
<td><em>Echiopsis</em> cortis</td>
<td><em>Elapogonathus</em> minor</td>
<td><em>Furina</em> diadema</td>
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<tr>
<td><em>Glyphodon</em> barnardi</td>
<td><em>G. dunalli</em></td>
<td><em>G. triatica</em></td>
<td><em>Heniaspis</em> damellii</td>
<td><em>Denisonia</em></td>
<td><em>Hoplocephalus</em> bistriatus</td>
<td><em>H. bubaroides</em></td>
<td><em>H. stephensi</em></td>
<td><em>Kerlaps</em> bimaculatus</td>
<td><em>Vermicella</em></td>
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<tr>
<td><em>Notechis</em> sternata</td>
<td><em>N. acutus</em></td>
<td><em>N. bicolor</em></td>
<td><em>N. juniperinus</em></td>
<td><em>Pseudolaps</em> multifasciata</td>
<td><em>P. pallidiceps</em></td>
<td><em>P. marmorata</em></td>
<td><em>P. ornata</em></td>
<td><em>P. serventi</em></td>
<td><em>P. erythraeomelas</em></td>
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<tr>
<td><em>Oxyuranus</em> acutus</td>
<td><em>O. scullectus</em></td>
<td><em>Parademania</em></td>
<td><em>O. microlepida</em></td>
<td><em>Pseudechis</em></td>
<td><em>austrius</em></td>
<td><em>P. colletii</em></td>
<td><em>P. guttata</em></td>
<td><em>P. perpunctatus</em></td>
<td><em>P. butleri</em> (Smith)</td>
</tr>
<tr>
<td><em>Pseudonaja</em> affinis</td>
<td><em>P. guttata</em></td>
<td><em>P. ingrami</em></td>
<td><em>P. modesta</em></td>
<td><em>P. nuchalis</em></td>
<td><em>P. textilis</em></td>
<td><em>Rhinocephalus</em> bicolor</td>
<td><em>Simoeaps</em> australis</td>
<td><em>Brachyophis</em> vernicellus</td>
<td><em>Rhynechotus</em></td>
</tr>
<tr>
<td><em>Suta</em> sutia</td>
<td><em>S. herboldi</em></td>
<td><em>S. fasciatus</em></td>
<td><em>S. incinctus</em></td>
<td><em>S. semicinctus</em></td>
<td><em>S. warro</em></td>
<td><em>S. sutia</em></td>
<td><em>Tropidechis</em> carinatus</td>
<td><em>S. spectabilis</em></td>
<td><em>Vernicella</em></td>
</tr>
<tr>
<td><em>Uenechis</em> breviscapus</td>
<td><em>U. carniolicae</em></td>
<td><em>U. flagellum</em></td>
<td><em>U. gouldii</em></td>
<td><em>U. monacha</em></td>
<td><em>U. nigrostriatus</em></td>
<td><em>U. gouldii</em></td>
<td><em>V. multifasciata</em></td>
<td><em>V. multifasciata</em></td>
<td><em>V. multifasciata</em></td>
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Table 1.1

This table lists on the left all species of Australian elapids recognized by Cogger, 1975 and 1979. Those species described since the publication of Cogger 1979 and indicated by the symbol (+) are listed after the other species of the genus. The classifications of eight other authors are also listed in columns under their names. Thus by reading from left to right one can determine each author's generic allocation of a species. Where a gap exists this indicates that the author's classification does not differ from Cogger's in that instance. Where the symbol (") is used this simply means the genus listed above is repeated. The table highlights the lack of unanimity among taxonomists utilizing classical morphological characters.
from the available morphological data. It is also apparent from this Table that many species have been assigned to four or more genera over the years. Likewise currently accepted generic divisions (Cogger 1975, 1979) may contain species that were at one time thought to be related to four different generic groups (see Simoselaps Table 1.1). The opinions of Cogger (1975 and 1979) have been used as a taxonomic guideline in these comparisons, and indeed throughout this thesis, because they represent the most recent and complete review of the Australian elapids and follow the basic guidelines of the International Commission for Zoological Nomenclature. Unfortunately Cogger's work still awaits the publication of his forthcoming checklist of Australian reptiles (1982 in press) for a detailed justification of some of the taxonomic allocations listed. Table 1.1 includes recent synonomies and lists species described since Cogger's 1975 publication. Because this Table is intended as a reference for the reader, only the major taxonomic discrepancies will be dealt with here.

A cursory examination of the Table indicates that many authors have used the work of Boulenger (1896) as a taxonomic guide to the Australian elapid fauna. Indeed, the subsequent reports of Worrell (1955, 1960, 1961, 1963), based largely on skull morphology, represent the first major shift since Boulenger's early work. Prior to his papers over 50% of Australian elapid species were assigned to two large genera, Diemenia (Demansia) and Denisonia. Worrell, however, attempted to reassign Australian elapid species into groups more closely reflecting similarities not only in cranial and dental characteristics but to a lesser extent in external
morphology and the limited understanding of behavioural and ecological criteria available at that time. This resulted in a division of Diemenia (Demansia) by the recognition of the genus name Pseudonaja for the brown snakes leaving only the whip snakes occupying the genus we now know as Demansia. Worrell then divided the large genus Denisonia into a series of genera including: Austrelaps for D. superba and signata; Cryptophis for D. pallidiceps (type), D. nigrescens, D. flagellum and D. dwyeri (= part of gouldii Cogger 1975, 1979), Drepanodontis for damelii, Drysdalia for coronata, coronoides and mastersi; Parasuta for gouldii and nigrostriatus; Suta for suta; and Unechis for carpentariae. He thus restricted Denisonia to the species devisii, fasciata, maculata and punctata. Worrell also divided the genera Pseudelaps and Furina as used by Boulenger 1896. Pseudelaps was dismantled by reviving the genus Brachysoma for diadema; describing a new genus, Lunelaps, for christianus; recognizing the genus Aspidomorphus in Australia for squamulosus alone; placing harriettae in the genus Glyphodon and redefining Cacophis to include kreffti. Boulenger has recognized Furina occipitalis, F. bimaculata and F. calonotus. Worrell described the new genera Nagrophis for bimaculata and Melwardia for calonotus and minima (= bertholdi).

As with any major change in taxonomic convention, Worrell's efforts solicited varied reactions. The most controversial move was the dismemberment of Denisonia. While most workers to date have accepted the use of Pseudonaja, Austrelaps (for superba but not signata) and Drysdalia they show differing attitudes toward the remaining genera.
Brongersma and Knaap-Van Meeuwen (1964) strongly opposed the splitting of Denisonia in their description of D. boschmai (later synonymized with D. (Unechis) carpentaria by McDowell, 1970). Based on a comparison with Worrell's figures and referring to the data of Kinghorn (1920), these authors demonstrated variation in two of the characters Worrell had used to define Cryptophis, and thereby rejected generic proposals of Worrell's division of Denisonia. Coventry (1971) avoided any mention of the generic classification of Worrell in his treatment of the black headed Denisonia of Victoria though stating this group was polyphyletic and similarities were due to convergence. Cogger (1975 and 1979) attempted to provide a general consensus of the relationships of species in this large group by critically applying the rules of taxonomic nomenclature. In doing so some of the generic names of Worrell were retained by consolidating in them species formerly allocated to Parasuta, Drepanodontis, Brachysoma Melwardia, Norophis and others (refer to Table 1.1 and see below).

As far as Denisonia is concerned, some authors (Storr, 1981 and Coventry, 1971) prefer to treat the problem in terms of species groups within a large genus. Though the distinction between species groups and genera may simply be a matter of semantics the definition of these species groups suffers from the restriction of reports to specimens whose ranges coincide with the geographic boundary of the State in which the study has been carried out. As a consequence, no complete treatment of the species groups involved has ever been produced.
The next investigator after Worrell to assess the relationship of Australian elapids was McDowell (1967). In a study of the New Guinea species of *Aspidomorphis* and their relatives he examined many Australian species and attempted to group them according to hemipene morphology and the structure of the abductor externus superficialis muscle surrounding the venom gland. In the Australasian elapid snakes, with the exception of *Elapognathus, Laticauda* and *Parapistocalamus*, the hemipenis lacks the alveolar calyces which are seen in many African and Asian forms (e.g. *Naja* and *Bungarus*).

The absence can, however, be explained in two ways yielding the following groups according to McDowell:

**Group 1** Most of Boulenger's *Denisonia* (Austrelaps, Drysdalia, Cryptophis, Hemiaspis, Suta and Unechis of Cogger 1975).

- Demansia
- *Pseudechis*
- *Pseudonaja* (except *P. guttata*)
- *Brachyaspis (= Echiopsis of Cogger, 1975)*
- *Oxyuranus*
- *Oqmonon*
- *Hydrelaps* (sea snake)
- *Aspidomorphis*

**Group 2** "Glyphodon Series" shows odd resemblance to *Ophiophagus*.

- *Denisonia maculata* and *devisii*
- *Acanthophis*
- *Hoplocephalus*
- *Glyphodon*

and Australian snakes assigned to *Aspidomorphis (= Cacophis and Furina of Cogger)*.

The classification of species according to the morphology of the abductor externus superficialis muscle results in somewhat different groupings:
Group 1. "Glyphodon type" abductor externus superficialis is considered primitive and is seen in most American elapids (Micrurus), Calliophis, African elapid genera (except Dendroaspis), Naja and most sea snakes and in the following Australasian forms:

- Glyphodon
- Furina
- Cacophis
- Vermicella*
- Neelaps*
- Simoselaps*
- Apistocalamus
- Toxicocalamus*
- Ultrocalamus
- Ogmodon
- Loveridgeelaps
- Elapognathus
- Drysdalia*
- Cryptophis*
- Pseudonaja*

Group 2. "Oxyuranus Type" of abductor externus superficialis includes Micrurus surinamensis and Dendroaspis which have developed this pattern independently. Hydrelaps (sea snake) is similar. Australasian forms include:

* Follows Cogger (1975) and subsequent authors for original list of species see McDowell 1967, p536 and 537.
Denisonia maculata
D. devisii
Acanthophis
Hoplocephalus
Solomonelaps*
Drysdalia coronata
Notechis
Tropidechis
Oxyuranus
Echiopsis

Group 3. "Pseudechis type" abductor externus superficialis is almost confined to Australasian terrestrial elapids but is also seen in Astrotia, Laticauda and Parapistocalamus.

Pseudechis
Micropechis
Unechis gouldi*, U. nigrostriatus, U. carpentariae, U. flagellum
Suta
Austrelaps
Denisonia punctata

Group 4. "Demansia type" abductor externus superficialis is most easily derived from the "Pseudechis type" and is seen in the sea snakes Laticauda schistorhynchus and Hydrophis (Ephalophis) mertoni as well as the following:

Rhinoplocephalus
Hemiaspis*
Demansia
Aspidomorphis

* Follows Cogger (1975) and subsequent authors for original list of species, see McDowell (1967, p. 536 and 537).
McDowell (1967) stated that, despite cases of parallelism and convergence, venom gland musculature "shows better correlation with other features than does any other single character". Using this character McDowell fully supports Worrell's separation of the genus Pseudonaja from Demansia. However in Worrell's division of Denisonia, McDowell found fault in the placement of "D" flagellum with pallidiceps and nigrescens in Cryptophis and suggested its association with gouldii, the type for Parasuta according to Worrell. This move is reflected in Coggers (1975) classification, though Unechis is used for gouldii and flagellum and Cryptophis retained for pallidiceps and nigrescens.

The reviews of the "Denisonia (Unechis) gouldii" species group in Victoria by Coventry (1971) and in Western Australia by Storr (1981) have resulted in redefining gouldii (restricted to W.A.) and dwyeri, (distributed in Victoria, N.S.W. and Queensland) as well as describing several new forms from Western Australia. Ehman (pers. commun.) has, for some time, been re-evaluating the species of Cogger's Unechis and their relatives. McDowell (1967) indicates a further anomaly in the inclusion of Denisonia punctata with the remaining species of Denisonia as restricted by Worrell. The venom gland musculature of D. punctata is more like that of the genera Suta, Parasuta and Unechis of Worrell; and this is in agreement with data on hemipenal morphology. In a later publication defining the "Pseudechis group" McDowell (1970) (see below) moved to include all those species in the genus Suta thus resulting in S. flagellum, S. punctata, S. fasciata, S. carpentaria, S. monachus and S. gouldii. This move has
not, however, been adopted by any subsequent author.

In his report McDowell demonstrated that *christianus* and *diadema* were congeneric. As the name *Brachysoma* which Worrell proposed was already assigned to a medusoid jelly fish, McDowell placed the two species in *Furina* (Durmeril 1853, type by designation *F. diadema*, Jan. 1859). This move eliminated the genus *Lunelaps* of Worrell. McDowell states "*Furina* is related to *Glyphodon* rather than to *Aspidomorphus*" (here presumably including *Cacophis*) or *Demansia*. *F. christianus* was included in *F. diadema* by Cogger (1975 and 1979). In a recent review of the genus *Furina* in Western Australia, Storr (1981) demonstrates the distinctiveness of *F. christianus* which he calls *F. ornata*. Storr (1981) also includes in *Furina* two species of the genus *Glyphodon* (*barnardi* and *tristis*) and one species of *Simoselaps* (*warro*) though no reassessment of these species is offered.

McDowell (1967) also analysed the species *kreffti*, *harrietae* and *squamulosus* which Worrell had placed in *Cacophis*, *Glyphodon* and *Aspidomorphus* respectively and, on the basis of hemipene morphology, dentition, head scutalation and color, combined them all into *Cacophis*.

In his report McDowell (1967) also points out that *signata*, placed in *Austrelaps* by Worrell (1963), could be identified as *Drepanodontis* using Worrell's (1961) key. In fact the venom gland musculature, dentition and skull morphology of *signata* is much like that of *Drepanodontis damelii* and McDowell places the two species together in *Drepanodontis*. Cogger (1975) has followed this but adopted the name *Hemiaspis* for the two species.
In a subsequent analysis of Australasian elapids McDowell (1970) defined two groups of genera: (1) the "Vermicella group" consisting of Vermicella (Sensu McDowell 1967, see below) within Australia, Salomonelaps, Loveridgelaps within the Solomon Islands and Ogmodon in Fiji; (2) the "Pseudechis group" including Micropechis in New Guinea, Pseudechis, Austrelaps (for A. superba only), Suta (for Suta Parasuta and Unechis of Worrell (1963)) as well as Denisonia fasciata and D. punctata and "Cryptophis" (Unechis) flagellum. These species groups and the ones described above, (McDowell, 1967), have been apparently elevated to the status of Tribes in the classification of Smith et al. (1977).

One of the most prolific contributors to Australian elapid taxonomy is Storr. In his 1967 work on Vermicella he combined species which Worrell (1960, 1961, 1963) and Kinghorn (1955) had earlier referred to the genera Narophis, Melwardia, Brachyurophis, Rhynechelaps and Rhinelaps. He also described several new forms. In a subsequent revision of the genus Storr (1979) elevated to species status four taxa, bertholdi, littoralis, anomala and minima, previously considered as subspecies of V. bertholdi and additionally described a new form V. approximans. Storr (1979) also referred warro to the genus Cacophis without any review of the characters but has subsequently chosen to place it in the genus Furina (see above, Storr, 1981). In his detailed analysis of the New Guinean genus Toxicocalamus McDowell (1969) showed that this genus was most closely related to the Australian genera Brachyurophis, Melwardia, Narophis, Rhinelaps and Rhynechelaps of Worrell 1963. McDowell recognized and agreed with Storr's (1967) move
to group these species together but excluded *annulata*. Since *annulata* is the type species for *Vermicella*, McDowell restricted the genus to *annulata* and proposed *Rhynchoelaps* (Jan 1758) as the next available name (type *Elaps bertholdi* Jan). The *Rhynchoelaps* group of McDowell (1969) therefore contains the two genera, *Rhynchoelaps*, which includes the *Vermicella* of Storr (1967) except for *V. annulata* and subspecies which have subsequently been elevated to species status (*multifasciata*), and the genus *Toxicocalamus* as redefined. Cogger (1975, 1979) has restricted *Vermicella* to *V. annulata* and *V. multifasciata* following McDowell (1969) but has placed most of the species of *Rhynchoelaps*, which he considers a "nomen nudum" (pers. commun.), in *Simoselaps* except for *bimaculata* and *calonota* which he refers to *Neelaps*. In a recent ecological study Shine (in press) follows Cogger's (1975, 1979) use of the genera *Simoselaps* and *Neelaps* but recognizes five species groups based on feeding habits, structure and scalation of the snout and dentition. The two latter characters Shine demonstrates to correlate with the first. Within the two genera Shine defines the following five distinct "species groups".

1. *Neelaps bimaculatus* and *N. calonotus* - saurophagus.

2. *Simoselaps "bertholdi"* group including *S. bertholdi*, *S. anomala*, *S. littoralis* and *S. minima* which were described originally as a subspecies of *S. bertholdi* by Storr (1967) but later elevated to full species status
(Storr, 1979). All are saurophagous and lack the exaggerated shovel-like snout of some other species as well as the dentition adaptations.

3. *S. "semifasciatus group" = S. semifasciatus, S. approximans, S. incinctus, S. roperi.* These species have a sharply upturned angular snout and are exclusively oophagous and possess a single enlarged triangular tooth at the back of the maxilla.

4. *Simoselaps australis* and *S. fasciolatus*, which Shine states may not be closely related, resemble *S. semifasciatus* but only *S. australis* shares the angular snout. Both are saurophagous and oophagous

5. *S. warro*, which Shine cites Storr (1979) as being so aberrant that it is only doubtfully included in this genus (see above for Storr's subsequent multiple assignments of this species).

"It should be noted that *S. warro* resembles the "*S. bertholdi" group in being saurophagous and lacking the modifications of the other species."

The genera of larger Australian elapid snakes have remained relatively stable since the "dissection" by Glauert (1948) and Worrell (1963) of the tiger snakes (*Notechis*) into a variety of subspecies and the recognition of *Austrelaps* and *Pseudonaja*. The review of the "black snakes" of the genus
Pseudechis by Mackay (1955) resulted in synonymising the species into the forms recognized today. In a recent comprehensive study of the Taipan, *Oxyuranus scutellatus*, and the small scaled snake, *Parademansia microlepidota*, Covacevich *et al.* (1981) analysed scalation, skull morphology, dentition, hemipene anatomy and karyotypes, concluding that these species are congeneric and referable to *Oxyuranus*. Apart from these few assessments of species, however, the intra and intergeneric relationships remain poorly understood. McDowell (1970) has associated Pseudechis and Austrelaps. The genus *Pseudechis* in Western Australia has recently been reviewed by Smith (1982) resulting in the description of a new species for the genus, *P. butleri*. Rawlinson has re-examined the monotypic genus Austrelaps and several species are likely to be recognized within it (pers. commun.). McDowell (1967) reported an unexpected resemblance between *Drysdalia coronata* and the genera *Notechis*, *Tropidechis* and *Oxyuranus*. Indeed he could find no internal character separating *D. coronata* and *Notechis*. Shine (1981) cites McDowell (1967) as indicating *D. coronata* may merit generic distinction. Storr is currently reassessing both *D. coronata* and *Notechis* (pers. commun.) and has synonymized all *Drysdalia*, *Austrelaps*, *Elapognathus* and *Echiopsis* under *Notechis* (Storr in press). The review of *Drysdalia* by Coventry and Rawlinson (1980) has resulted in a redefining of *D. mastersi* and *D. rhodogaster*. Storr (1981) has also reviewed the death adders (*Acanthophis*) of Western Australia and re-described a third species, *A. praelongus*. 
In addition to these morphological criteria the possible relationships indicated by venom characterization and immunological studies are of interest. Unfortunately, data is generally only available for the species of larger Australian elapids due to the relative difficulty in obtaining venom and blood samples from the smaller varieties. Minton and da Costa (1975) indicated close affinities between sea snakes and the two elapids Denisonia (Austrelaps) superba and Notechis when their venoms were cross reacted to other venoms. They also pointed out that Notechis venom did not react with Denisonia (Austrelaps) antiserum though these gross measures were intended to determine the relationship of these terrestrial elapids to sea snakes and not the intergeneric relationships of the Australian forms. In a more recent study, Minton (1981) analysed serological data from 11 genera native to the eastern New England region of NSW and found Pseudechis, Pseudonaja and Tropidechis to be closely related while Acanthophis, Demansia and Vermicella were somewhat remote from the preceding group. No comment was made on the interrelationships of the latter three genera. Coulter et al. (1981) examined in some detail the venoms of Pseudechis, Pseudonaja, Austrelaps, Acanthophis, Oxyuranus and Notechis, as well as Asian terrestrial elapids and the sea snake Enhydrina, by enzyme immunoassay (EIA) and concurred with Minton and da Costa (1975) that the venoms of sea snakes were closely related to that of Australian snake venom but not to that of the Asiatic elapids. The venoms of several Australian species differed greatly in respect of each other and it is interesting to note that in the reaction of species to anti-Notexin (from
Notechis), as assayed by EIA, Austrelaps gave a reaction most similar to Notechis itself. Morrison (pers. commun.) has found great similarities in the venom characteristics of Tropidechis and Notechis with an immunological cross-reactivity of greater than 50%. Studies underway on the coagulant properties of species of Pseudechis indicate that P. porphyriacus is somewhat distinctive in venom compared with the other species (pers. commun.). The venom studies of both Saint Girons and Detrait (1980) and Southerland (1979), point to a close similarity of the venoms of Oxyuranus and "Parademansia" (now = Oxyuranus) and their distinctiveness from Pseudonaja. Saint Girons and Detrait (1980) go on to demonstrate that the venoms of Austrelaps and Pseudechis possess many common antigens and they suggest that, with Bungarus, it occupies "a central position among elapines of the Old World". They conclude that Australian genera do not form a homogeneous group in that the venoms of many species share antigens with Bungarus, whereas Oxyuranus, Parademansia (=Oxyuranus) and Pseudonaja have very weak cross reactivity with other elapids.

It should be recalled from the previous section that the MC'F work of Cadle and Gorman demonstrated that Demansia was distinctive among Australian elapids in displaying the greatest immunological distance from all the elapid reference species they used and thus may be phylogenetically distinct.

From the limited chemotaxonomic data several inadequacies are evident. Firstly, with the exception of the preliminary report of Minton (1981), none of the phylogenetic studies utilizing MC'F and immunodiffusion were initiated within
Australia. The sample species studied were consequently very limited. As for the venom analysis, only one report, that of Saint Girons and Detrait (1980), appears to have been initiated to elucidate phylogenetic relationships and it, like those studies within Australia, appears limited to species containing large specimens from which adequate venom samples are more commonly available.

This review of morphological characters, and the classifications derived from them, demonstrate that only two (Rhinoplocephalus and Hoplocephalus) of the 25 genera of Australian elapid snakes have not been altered taxonomically in the past two and a half decades. This reflects the small measure of agreement on intergeneric relationships. The following six questions appear to me to be the most important in attempting to resolve this situation:

1. Do the Australian elapids represent a homogenous assemblage and if not, is there evidence for a polyphyletic origin by multiple invasion?

2. What are the probable phylogenetic relationships among the larger Australian elapid snakes dangerous to man?

3. Species within the genera Acanthophis (Storr, 1981), Pseudonaja (Gillian, 1975, Cogger, 1975) and Furina, clearly appear to be composite. Does chromosome banding analysis allow us to distinguish novel forms within the currently recognized groups?
4. What are the relationships between the "species groups" and genera making up the "Denisonia" of Boulenger and does a comparison of morphological and chromosomal data allow a more reasonable grouping of these species and genera?

5. Do the tribes listed by Smith et al., 1977 and defined by McDowell most accurately represent suprageneric relationships?

6. Can chromosome data be helpful in resolving the relationships of particularly unusual species (e.g. Simoselaps (V.) (C) (F) warro)?

In an attempt to answer some of these questions and those proposed in the preceding section, chromosome banding data have been collected on a large variety of species and have been analysed within the parameters proposed in the next section on cytotaxonomy and chromosome evolution.

1.2 A Cytological Overview

1.2.1 Cytotaxonomy, Chromosomes and Evolution

Cytotaxonomy can be defined as the use of chromosome analysis to detect patterns of shared fixed differences in chromosome constitution between groups of organisms in order to derive a phylogeny presumed to more closely reflect the genetic relationships of those forms. This approach is predicated
upon the assumption that the variety of karyotypic differences observed between extant species are in some sense indicative of the relationship between chromosome change and the speciation process. It thus also assumes that the karyotypic relationships that can be demonstrated between taxa can be used as supportive evidence of the relationship of the taxa concerned.

As we shall see from the discussion below, there are two distinct approaches to the use of chromosome data to interpret taxonomic status (Fig. 1.1).

The first is a horizontal approach in which shared fixed differences are used to group extant forms. When homologies within groups can be assuredly demonstrated, and between group differences clearly defined, this exercise can be valuable in delimiting species groups and defining sets of related taxa in general.

The second and more tenuous approach is the extrapolation of the data gathered from extant forms to derive a phylogeny or chronological lineage.

There has always existed a school of thought that most, if not all, chromosome changes between species are essentially neutral in character and hence are coincidental to the speciation event rather than causal (see Ohno, 1974). However, even the exponents of this view do not deny that the conditions that promote speciation may also lead to the fixation of chromosomal variants and so, regardless of their neutrality, may indirectly offer a convenient marker for assessing species relationships and indeed be of value in constructing phylogenies. The alternative point of view, that
Figure 1.1  The use of chromosome data to assess relationships between taxa is illustrated by this diagram. As outlined in the text (section 1.2.2) this can be a two step process. The horizontal analysis involves the assessment of banding data to determine shared fixed differences between extant species. The allocation of species to a karyomorph group based on shared fixed differences in its karyomorph can be valuable in constructing groupings of taxonomic significance. The second and more tenuous stage of the analysis is the construction of a phylogeny based on presumed interrelationships of the karyomorph groups in relation to the ancestral karyomorph. The procedure used in determination of this primitive karyomorph is outlined in the text.
Shared Fixed Derived Differences

Geologic Time

Present day

Extant Taxa

Ancestral Karyomorph

Extant Species Groupings

Phylogenetic Analysis
chromosome change may be causally related to speciation by
serving as a reproductive isolating mechanism, rests upon the
fact that what chromosomes do at meiosis is fundamental to
fertility and hence to survival. Chromosome differences which
impair meiotic pairing or meiotic segregation both lead to
infertility. Such disturbances may arise in interspecific
hybrids because of structural differences between the
chromosome sets of the species involved. This implies that
these structural differences may have been instrumental in
acting as primary reproductive isolating mechanisms during the
speciation process.

A complication frequently raised in dealing with the
question of the significance of chromosome differences which
differentiate related species is the issue of whether
chromosome change itself leads directly to morphological change
of a kind likely to serve as a basis for taxonomic
distinction. There are relatively few cases where chromosome
change has been correlated with morphological change. The two
most obvious ones which come to mind are:

(1) The relationship in the white throated sparrow
Zonotrichia between certain sex linked plumage
characteristics and a polymorphism for a pericentric
inversion system (Thorneycroft, 1966, 1975) which has the
effect of leading to negative assortive mating. This
relationship, however, operates within a species and, as
is well known, the conditions which lead to polymorphisms
of this sort need not lead to species differentiation.
(2) The parallel which King reports (1976, 1981) between back pattern differences and the karyotypic differences which distinguish between chromosome races of the geckoes *Diplodactylus vittatus*, *Phyllodactylus marmoratus* and *Gehyra variagata*. Even here it remains to be demonstrated that the pattern differences are determined by the chromosome differences which, in this case, are thought to involve centric fusions.

Thus we see that the difficulty confronting those who wish to utilize chromosome data in a broad evolutionary sense lies not only in the demonstration of the nature of the particular chromosome change and its possible effects on speciation, but also in attempts to correlate these changes and compare the patterns of fixed differences with classical taxonomies which are derived from morphological comparisons alone. Clearly we cannot expect a simple and direct correlation between chromosome change and morphological change. This does not negate the use of these data but perhaps indicates that one must be more discerning than often has been the case in the past.

Atchley (1972), for example, has drawn attention to the difficulty of using data on simple chromosome number and morphology to infer lineage relationships and phylogenies since such data does not offer a rational means of defining genetic homology. Yet accurate information on homology of genomes is mandatory to both the "horizontal" analysis, or grouping of extant forms, and in determining the direction of chromosome change when constructing phylogenies. Gross karyotypic structure does
not define genetic homology. It is precisely for this reason that the karyotype is defined in terms of the phenotypic appearance of the chromosomes rather than their genetic content. Yet the majority of past workers on snake cytology have been perfectly content to use gross karyotypic data in this simplistic way.

Karyotype comparisons also necessitate assumptions concerning the direction of evolutionary change. Unfortunately the ready reversibility of many types of chromosome change make detection of their direction no easy matter. This is particularly apparent from gross karyotypic studies of lizards where various workers studying the same groups have interpreted chromosome number changes to be due either to fissions (in *Sceloporus*, Gorman et al., 1969; in *Cnemidophorus*, Lowe et al., 1970; and in *Amphisbaenidae*, Gorman et al., 1973) or fusions (in *Sceloporus*, Cole, 1970; Hall and Selander, 1973; in *Cnemidophorus*, Lowe et al., 1970b; and in *Amphisbaenidae*, Huang and Gans, 1971) depending upon their assumptions concerning the presumed ancestral karyotype.

The only direct means of assessing chromosome homology in cytological terms, is through an analysis of meiotic pairing behaviour in interspecific hybrids, though this is not without complications (John and Lewis, 1965). Due to the presence of prolonged sperm storage in females, and a relatively long period to maturation, hybrid studies are not as yet practicable in reptiles. There is, however, an alternative approach which relies on the use of mitotic chromosomes which, when treated with various proteolytic agents, appear transversely banded. This "G-banding technique", which has been widely used in human
and other mammalian cytogenetic studies, appear highly reproducible and gives each segment or arm of the chromosomes a specific banding character. This allows each chromosome in the genome to be identified and even minor changes in banding pattern between genomes detected. The assumption here is that the bands reflect true genetic homology. Where it has been possible to test this, as in the X chromosome of mammals, there is a clear correlation between conservation of G-bands and gene content (compare Pathak and Stock, 1974 with Ohno, 1974). This has been confirmed in cases where G-banded autosomal segments pair at meiosis in hybrids (Elder and Pathak, 1980) though the paper by Nesbitt (1974) cautions against a priori acceptance of such a direct relationship.

An additional means of detecting changes in chromosome structure which is not apparent from a comparison of gross karyotypes, is the treatment of the cell with an alkaline base. This technique of "C-banding" allows the detection of constitutive heterochromatin in somatic cells. As we shall see from the data presented in this thesis, there exist a number of distinct forms of constitutive heterochromatin in terms of their different behaviour when exposed to various banding and fluorescence treatments. Although the distribution of C-banding material is known to be highly variable, its cytotaxonomic significance is evident when large blocks of heterochromatin are found to be responsible for changes in the phenotypic character of the gross karyotype. As heterochromatin is known to be a class of material depauperate in genes, and in which changes in amount can occur rapidly by amplification or unequal exchange, the
identification of such large heterochromatic blocks is essential to the accurate interpretation of differences in gross appearance between karyotypes.

Mengden and Stock (1978) have made a strong case for the use of C- and G-banding analysis in phylogenetic studies of snakes by demonstrating the use of these techniques for detecting errors in gross karyotypic interpretation. Previous errors in sex chromosome identification, for example, were detected by using C-banding, as were errors in the interpretation of structural changes (inversions, whole arm heterochromatic additions, and micro to macro-chromosome conversion) by comparing both C- and G-banding data (Mengden and Stock, 1980). From an analysis of G-band patterns of several groups of non-mammalian vertebrates, the same authors (Stock and Mengden, 1975) have demonstrated a general conservation of G-band segments within groups, while stressing that high quality G-banding must be attained before accurate comparisons of data between species is possible. Indeed, Haiduk et al. (1981) state that gross karyotypic data underestimated the magnitude of chromosome change in African megachiropteran bats by a factor of 4.5 when G-band data was compared. Fig. 1.2 illustrates the major types of chromosome change which are known to occur as fixed differences between species of snakes. Comparable cases are well known in other groups which have been more extensively studied.

From this illustration (Fig. 1.2) some of the shortcomings of gross karyotypic analysis and the advantages of banding analysis, are immediately apparent:
Figure 1.2    The following three pages of illustrations depict some basic chromosome structural rearrangements that are more easily and accurately detected by employing G and C-banding techniques. A detailed discussion of the advantages of these banding techniques over conventional gross karyotypic data can be found in the text section 1.2.1.
Pericentric Inversion

Paracentric Inversion

Centric Transposition

G-band Appearance

centromere and
G-band inverted

G-band inverted but
centromere not moved

centromere moved but
G-band unchanged

Centric Shifts
Gross Karyotypic Phenotype
Robertsonian Rearrangements

Gross Karyotypic Phenotype

Fission

Fusion

Any two elements of similar size & centromere position could be involved

G-bond Appearance

Tandem Fusions

Gross Karyotypic Phenotype

Fission

Fusion

Precise chromosome segments involved can be identified

G-bond Appearance

Inactivation of one centromere
Whole Arm Heterochromatin Additions

Gross Karyotypic Phenotype

C-band Appearance

or Interstitial
1. Gross preparations cannot distinguish between pericentric inversions (PI) which alter G-band sequences and centric transpositions which need not alter sequences. Such a centric transposition which was formerly assumed to be a P.I has been reported in the snake *Elaphe subocularis* by Mengden and Stock (1980) using G-band data.

2. Paracentric inversions are not detectable in gross karyotypes. However no such rearrangement has to date been reported from the limited G-band studies made on snakes.

3. Chromosomes contributing to fusion/fission events can only be identified by relative length and centromere position in gross preparations. Consequently homologies between the chromosomes involved in fusion as well as the fusion events themselves cannot be reliably identified. G-band data can precisely identify the chromosomes involved and the homology of products between genomes.

4. Tandem fusion products and large heterochromatic additions could appear similar in different taxa when gross karyotypic data alone is used. Such large, whole arm, additions have been reported in *Sanzinia* by Mengden and Stock (1978, 1980).

5. Likewise small whole arm additions of heterochromatin are indistinguishable from pericentric inversions in gross karyotypic preparations when they do not result in a radical difference in overall length.
6. Whole arm heterochromatic additions which result in the chromosome changing from acrocentric or subacrocentric to metacentric could easily be confused with a fusion or an inversion event when gross karyotypic comparisons are made between species. Such whole arm additions have been reported in several species of snakes (Mengden and Stock, 1978, 1980 and Mengden, 1981).

In addition to the structural changes described above, there remain two broad classes of chromosome variation which have been used by some authors as cytological markers between species. These are the variation in sex chromosome morphology and the variability in location, and definition of nucleolar organizer regions.

Since the discovery of heteromorphic sex chromosomes in snakes (Kobel, 1964) they have been found to be the most variable elements in the serpent genome. Becak and Becak (1969) and Singh (1972, 1974) have claimed that there is a parallel between sex chromosome differentiation and morphological specialisation in snakes and have then used the degree of sex chromosome differentiation as a cytotaxonomic tool by arguing for a sequential relationship in sex chromosome differentiation. In this thesis the nature of variation in, and composition of, the sex chromosomes is analysed with a variety of techniques, including in situ hybridisation, fluorescence and banding to determine (1) the underlying structural variation; (2) at what taxonomic level specificity can be demonstrated, and (3) whether any detectable sequential relationship can be used as a tool in constructing
phylogenies. In short, to what degree can sex chromosome differentiation in snakes be used either as a phylogenetic indicator or to provide useful information on the general mechanism of sex chromosome evolution.

The characterization and location of nucleolar organizer regions (NOR's) have also been used as markers in cytotaxonomic studies, particularly in anuran amphibians (Early, 1971; Schmid 1978, and King, 1980). Secondary constrictions, often associated with NOR's, have not been observed in the karyotypes of the majority of snake species that have been analysed. However they are apparent in the karyotypes of many sea snakes (Singh, 1972, 1974) and in the Australian elapids (Shine and Bull, 1977; Mengden, 1981). Becak and Becak (1972) have demonstrated the presence of "satellite" type secondary constrictions on chromosomes from embryonic tissues when such constrictions are not visible on chromosomes from adults. Such ontogenetic variation complicates the interpretation of these types of data; consequently in this thesis a variety of techniques are applied to characterize the variation in NOR's and determine its possible significance from a cytotaxonomic viewpoint.

As a prerequisite of any consideration of karyotypic evolution one needs to be able to define a primitive snake karyotype and here one meets with considerable conceptual difficulty. The conventional way out of the paradox, as we shall see from the following section, has been to apply the principle of parsimony using the assumption that the most common condition is likely to be the primitive condition. Many workers have accepted Becak's (1965, 1969) proposal that
the primitive snake karyotype possesses a diploid number of 36 including 16 macrochromosomes and 20 microchromosomes because such a karyotype is both common and is found in the "primitive" boids. Such an *a priori* assumption is clearly oversimplistic for two reasons: (1) it assumes that forms judged to possess "primitive" morphological characteristics are most likely to exhibit "primitive" karyotypic character, and (2) it assumes direct homology between the similar "primitive" gross karyotype reported to be common in a number of different families. No careful study of the true homology between gross karyotypes of different families sharing the same diploid number and configuration has been conducted in snakes using modern techniques or hybridization. Indeed Mengden and Stock (1980) showed that, though some degree of homology in banding patterns existed within, and to a lesser extent between, genera of boids there were also examples of broad divergence in banding patterns. How then can one detect an archetypic karyomorph in snakes where an appropriate fossil record does not exist? If banding homologies can be demonstrated it is more reasonable to assume that diverse and obviously unrelated forms sharing the same karyomorph are conserving an ancestral condition than it is to assume they might have derived such a condition by convergence and resulted in precisely the same banding pattern. It should be obvious that conservation of a primitive character does not denote relationship between forms. In order to sensibly group organisms we must look first at derived fixed differences. Such a tempered parsimony might be expected to be accurate within the limitations of the elapid radiation. On the other hand, a broader analysis of
the suborder Serpentes would be more difficult because of the broad homologies that would have to be demonstrated.

1.2.2 Cytotaxonomy of Snakes

Table 1.2, to my knowledge, lists all species of snakes for which there is chromosome data available. It also includes the data which I have obtained on approximately 100 taxa not previously reported, 82 of which are elapids.

Thatcher (1922) was the first to report chromosome number in a snake. From histological sections of testes he identified the diploid number of *Thamnophis* (= *Thamnophis*) butleri as 37 and explained the uneven number in terms of an XXY sex determining mechanism. This was later found to be inaccurate (Ogumo and Makino, 1932) and we now know that *Thamnophis* shares a typical ZW female heterogeneity exhibited in many snake species with identifiable sex chromosomes (Baker et al., 1972). Using similar histological techniques to Thatcher, several workers began to describe chromosome number differences in snakes (Matthey, 1928, 1929, 1931; Nakamura, 1927, 1928, 1929, 1935; Ogumo and Makino, 1932). These data were organized in a systematic fashion and first reviewed by Matthey (1949) and Makino (1951). Due to the inadequacies of the techniques of testicular sectioning, both of these accounts have subsequently been shown to contain errors.

By 1951, while representatives of many families had been studied, there had been no attempts to study variation within genera. The 1950's showed little advance in the technology of handling snake chromosomes. The one major finding during this period was the demonstration that the male snake possessed
Table 1.2  The following ten pages lists diploid number, macro and microchromosome numbers and sex chromosome details (wherever available) for all species for which chromosome data is available. It also lists the pertinent literature citations. I have refrained from listing species for which data has not as yet been published except where I have direct knowledge. For further discussion see text section 1.2.2.
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*Note: The species listed are from the Colubridae family, and their references are from various sources as indicated.*
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*Notes:*
- M: Male
- A: Adult
- St: Stomach
- 3m: Third mitotic figure
- M, m: Male, Female
- M: Adult
- M, m: Male, Female
- St: Stomach
- Yes, No, Details:

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- Dürren, 1978
- Rossman and Parlee, 1977
- Dutt, 1970
- Dutt, 1970
- Tuh, et al., 1970
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Cratalus adamantus
  C. siro M F
  C. laevissimus F
  C. durissus durissus
  C. g. terricinus M F
  C. horridus F
  C. lepidus F
  C. mitchelli
  C. polius M F
  C. ruber
  C. scutulatus M
  C. terrificus M
  C. viridus latusus
  C. y. organism
  C. y. viridis M F
  C. oerastes F

Lachesis muta ziemaphrya
  L. m. muta M F
  Sistrurus milliusus
  S. catenatus M

Trimeresurus flavoviridis M
  T. gramineus M
  T. macroagmatus M
  T. okinawensis M

Viperinae

Chic carinatus F
  C. carinatus M

Vipera aspis aspis M F
  V. a. zinnkerti M F
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  V. lebetina M F
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  V. a. latastei latastei M F
  V. a. monticola M
  V. secanal M F
  V. a. armadylus M
  V. a. montadoni F
  V. secanal F Y. a. zinnkerti M

Fischman unpublished
Baker et al., 1972
Baker et al., 1972
Gutierrez et al., 1979
Bozak 1965
Baker et al., 1972
Zimmerman and Kilpatrick 1973
Baker et al., 1971, 1972
Johnson unpublished in German 1973
Baker et al., 1972
Johnson unpublished in German 1973
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Monroe, 1962
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Makino and Norina, 1949
Nakamura, 1935
Nakamura, 1935
Makino and Norina, 1949
Singer et al., 1970, Brack 1980
DeSmet, 1978
Kobel, 1967, Kobel, 1963, Matthey, 1931
Kobel, 1967, Saint-Sirates, 1777
Kobel, 1967, Kobel, 1962, Saint-Sirates, 1777
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Singer, 1974, Singer et al. 1170
Kobel, 1967, Saint-Sirates 1777
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Mengden present study, Fischman unpublished
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RayChaudhuri et al 1970, Singh et al 1970a,
RayChaudhuri and Singh 1972, Mathew 1949
Singh et al 1970
Singh 1972
Nakamura 1935
Singh et al 1980
Mengden present study
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Gutierrez and Bolonos 1979
Graham 1977
Beek and Beek 1969
Gutierrez and Bolonos 1979
Gutierrez and Bolonos 1979
Gutierrez and Bolonos 1979
Dafnet 1978
Mengden present study
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Mengden unpublished
Mathew 1949
Nakamura 1935, Singh et al 1970
RayChaudhuri et al 1971, RayChaudhuri and Singh 1972
Mengden present study
Dutt 1966
Singh et al 1940
Dafnet 1978
Mengden present study
Mengden present study
Mengden present study
### Australian Terrestrial Elapids

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<td>Gutierrez and Bolanos 1980</td>
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<td>Gorman 1980</td>
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<td>Wakahara 1995</td>
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<td>Gorman 1991</td>
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<td>Gorman 1991</td>
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In his review Matthey (1949) had established the use of fundamental number (NF) by assigning a value of 1 to each chromosome arm. Thus metacentric or biarmed chromosomes were given a number of 2, while acrocentrics and microchromosomes had a value of 1. By comparing the NF between species which differ in diploid number many species could be shown to conserve the number of chromosome arms, so maintaining the same NF despite showing variation in diploid number. Using this interpretation Kobel (1963, 1967) was able to demonstrate possible homologies between the chromosomes of *Vipera berus* (2n = 36) and *Vipera aspis* (2n = 42).

Subsequent advances in the study of snake chromosomes have been closely tied to technological improvements. The adaptation of human blood culture techniques by Becak and his colleagues in the early 1960's (Becak et al., 1962, 1963) represented a major technological advance. Using this technique Becak and Becak (1969) added information on chromosomes in an additional 15 species and reviewed the data on the 87 species published to that time. From their limited sample sizes within these 15 species these authors concluded that "the morphology of the chromosomes and the amount of genetic material does not vary among individuals of the same species; differences from the species standard are generally due to chromosomal aberrations" (Becak et al. 1968). Although this opinion has been perpetuated by many subsequent workers, adequate samples still do not exist to judge its validity.
They also stated that the variation in chromosome number of both macro- and microchromosomes could be explained by Robertsonian fusion events without appreciable change in DNA content (Becak and Becak, 1969; Becak et al., 1964a; Atkin, et al., 1965). As we shall see, this too is an oversimplistic interpretation.

Additionally the Becaks for the first time proposed that the archetype or ancestral karyotype in serpents was one with a diploid number of 36. This basic karyomorph included 16 macrochromosomes and 20 microchromosomes with a NF around 50 which they observed in the majority of species karyotyped to that time and in those forms (i.e. boids) which were generally considered morphologically 'primitive'. This idea too has been perpetuated by subsequent workers in the field.

The post-60's saw a flurry of activity in snake chromosome cytology. Fischman examined blood culture and testicular preparations of 11 species for his PhD dissertation, though all of his data remain unpublished except as abstracts. Bury et al. (1970) contributed gross karyotypic data for 12 species of North American colubrid snakes, though these authors relied heavily upon techniques utilizing testicular material and so were unable to report sex chromosome occurrence and morphology. Itoh et al., (1970) on the other hand, utilized both blood and tissue culture to detect sex chromosome dimorphism in five species of Japanese snakes. Gorman and Gress (1970) and Singh et al. (1968) utilized the injection of a mitogen for spleen preparations and blood culture techniques to study the cytotaxonomic relations of some boid species.

Baker et al. (1971) published the first in vivo technique
involving the interperitoneal injection of phytohaemoglatinin at 24 hr intervals prior to injection of a vinblastin sulphate derivative as a mitotic arresting agent. Blood could then be drawn from the snake for karyotyping without the necessity of sacrificing valuable specimens. Utilizing this technique, Baker et al. (1972) contributed data on 38 species of North American snakes which represented the largest survey to that date. The interpretation of the chromosome data on natricine snakes reported in this paper, coupled with that of Eberle (1972), has resulted in their reclassification so as to reflect the taxonomic dichotomy between New World and Old World natracines as we understand them today (Rossman and Eberle, 1977). This was the first time in snakes that cytological data had resulted in a formal reassessment of taxonomic status.

A modification of the in vivo technique of Baker et al. (1971) subsequently resulted in a microharvest method that allows analysis of very small specimens (Branch, 1978). It has also led to the development of an in vitro culture method involving the exposure of blood to colchicine after in vivo stimulation with PHA and so further safeguards valuable specimens (Taylor and Bolanos, 1975). The application of the latter technique has allowed Gutierrez et al. (1979b) to contribute gross karyotypic data on 10 species of Viperidae and 4 species of Elapidae (Gutierrez and Bolanos, 1979).

Almost simultaneous with the publication of Baker et al. (1972), Singh (1972b) published data on 17 species of Indian snakes and reviewed data on the 110 species reported in the literature known to him (but exclusive of Baker et al., 1972 and others in press concurrently). Singh's work described sex
chromosome evolution in snakes, established the common occurrence of multiple sex chromosomes in female hydrophid sea snakes (see also Singh, 1972a) and made numerous taxonomic suggestions based on the gross karyotypic data reviewed.

Trinco and Smith (1972) also published a review of ophidian karyology at this time. It suffered, however, from the fact that it could not include the major works mentioned above which were published simultaneously. As this review contributed no new data, it became immediately obsolete. A similar fate was avoided by Gorman (1973) with the inclusion of an addendum to his review. This remains the most recent, thorough assessment of snake chromosome data, though a computer compiled list of species, chromosome numbers and complete authors citation was published the following year by Gilboa (1974) and a supplement update to Gorman's review has recently been published by Peccinini-Seale (1981). Singh (1974) analysed an additional six species of Indian snakes and concluded that the lack of similarity between the karyotypes of the Elapidae and of the Hydrophidae justifies the controversial separation of these taxa.

In 1975 G- and C-banding techniques were first adapted and applied to snakes as well as other classes of non-mammalian vertebrates (Stock and Mengden, 1975). As predicted by Gorman (1973), these two technological advances have resulted in an interest in reptilian material not only for cytotaxonomic studies, but to investigate chromosome structure as well. This is particularly apparent in the investigation of sex chromosome evolution (Becak and Becak, 1981; Bull, 1978, 1980; King and Rofe, 1976; King 1977; Mengden and Stock, 1978, 1980;
Mengden, 1981; Singh et al., 1976, 1979, 1980; Sites et al., 1979a).

In 1977 Saint Girons examined a large variety of European *Vipera* species to assess the variation and evaluate Kobel's (1967) fusion model to explain the relationship between 2n = 36 and 2n = 42 species. This was the first cytological study of snakes to utilize meiosis of hybrid specimens. All hybrids possessed 2n = 39.

The largest gross karyotypic survey of snake chromosomes to be published since 1972 is that of De Smet (1978) who reported data on 23 species. Of these, however, only eight had not been previously published. The poor quality of some preparations and the discrepancies with other published figures, leads one to suspect technical difficulties, misidentification of some specimens, or discrepancies in nomenclature in this report.

Gutierrez and Bolonos (1979) published data for four elapid taxa demonstrating variation in diploid number and NF as well as in the number and morphology of macro and microchromosomes. This high degree of variability parallels that found in other species of this family, while the 10 species of Viperids they studied (Gutierrez et al. 1979) shows relative conservation, as is characteristic of other species of this family reported thus far.

The most recent contribution to snake cytotaxonomy is that of Gorman (1981) who reported gross karyotypic data for three species of laticaudine sea snakes and reviewed chromosome data for 18 species of elapids (excluding DeSmet, 1978). He concluded that most variation could be attributed to centric
fission and sex chromosome rearrangements. A similarity in
gross karyotype between Notechis (2n = 34; 14M, 20 m), Shine
and Bull (1977), and one of the three Laticauda species he
studied caused him to suggest that this karyotype may be
primitive for the elapid radiation. In the light of the high
degree of variation in the chromosomes of other Australian
elapids demonstrated in this thesis such an assumption is quite
untenable.

1.2.3 Conclusions and Caveats

In the preceding introduction I have attempted to outline
for the reader both the advantages and the reservations or
restrictions involved when chromosome data is utilized to
determine phylogenetic relationships.

The following observations express the limitations of
previous attempts to use a cytotaxonomic approach to the study
of snakes:

1. Much of the earlier work on snake chromosomes utilized
testicular material to obtain gross karyotypic data. Not
only does this preclude the use of sex chromosome morph-
ology in the analysis but as recently as 1978 preparations
of too poor a quality to be valuable for detecting even
gross chromosome differences have appeared in print.

2. Less weight has been applied to microchromosome evolution
because of poor resolution of these elements. They have
all been treated essentially as uniarmed, which in reality
is not the case.
3. The direction of change in both inversions and fusion-fission events is often difficult to ascertain with confidence and is usually determined by divergence from a presumed ancestral karyotype.

4. Most workers assume that the commonest karyotype observed in the greatest number of taxa, and from which most simple derivative karyotypes can be produced, is the primordial type.

5. Workers tend to look for the least number of rearrangements to get from the commonest karyotype to the most divergent. This assumes that changes are due to a series of single rare events and that change and phylogeny are both linear progressions. Indeed the assumption of the unique origin of the sequential steps in the phylogeny and the homology within groups is basic to the construction of phylogenies.

6. In comparisons involving present day species we are looking at the products of evolution and not the process itself. This does not necessarily tell us anything objective about the phylogenetic events which have led to the present day.

7. The final caveat must be that while certain taxa are conservative in gross karyotypic morphology others, including the elapids, are highly variable. As pointed out by Gorman (1981) when an entire family is variable there is no reason to assume "a priori" that species and
even populations are not variable, yet it is assumed that
the relatively few individuals studied represent the popu-
lation. Though the present study represents the largest
survey within a snake family and in some species the
largest sample sizes examined to date, this caveat must be
kept in mind when reviewing the examples of intraspecific
variation.
CHAPTER 2
2.1 Materials

Specimens were either collected from wild populations or else obtained from zoological collections and exhibits. In the latter instance precise locality data were not always available. Table 2.1 lists sex and locality data, where available, for all specimens examined. Recent technical improvements have made it unnecessary to sacrifice specimens in order to obtain data. Those specimens that were sacrificed have been deposited, with reference to the catalogue numbers listed in Table 2.1, in major museums within Australia. The bulk of voucher specimens are housed in the Australian Museum collection at Sydney or the National Collection, CSIRO, Wildlife Division, Canberra. Where State law requires it, voucher specimens of species collected within a State have been deposited within the respective State museum. The author accepts responsibility for the identification of all taxa. Verification of species identification, where necessary, was obtained with the assistance of R. Jenkins, N.P.W.S.; J. Wombey, CSIRO, Wildlife Division, and Museum personnel upon deposition of voucher specimens.
Table 2.1 The following five pages constitute locality data for the specimens from which chromosome data was obtained for this thesis. Where locality data is not available for a particular animal the specimen number is followed by the letters N.L. followed by the name of the individual or institution that houses that specimen.
Specimens Examined

African and Asian Elapids

Aspidelaps lubricus (144) F N.L. San Antonio Zoo
A. scutatus (331) F N.L. Australian Reptile Park/ Worrell
Boulengerina annulata (355) N.L.

Dendroaspis polylepis (167) F N.L. Houston Zoo

Hemachatus haemachatus (304) M N.L. Australian Reptile Park/ Worrell +2

Naja melanoleuca (139) F N.L. Houston Zoo
N. naja kaouthia (308) N.L. Australian Reptile Park/ Worrell
N. n. polyocellata (356) N.L. Dallas Zoo
N. nigricollis (140, 141) M F N.L. San Diego Zoo
N. haje (138) F N.L. San Diego Zoo (300, 301) N.L. Taronga Park Zoo

Ophiophagus hannah (145) M N.L. Houston Zoo, (305) M N.L. Australian Reptile Park

Walterinnesia aegyptia (143) M N.L. San Antonio Zoo +1

Sea Snakes

Aipysurus duboisi (153) M Coral Cay, Marion Reef, Coral Sea (187) M N.W. Shelf
A. fuscus (333) N.L. Cogger/Moffit
A. leavis (188) Swains Reef, Coral Sea (189) " " " "

Disteiria major (282) F Coral Cay, Marion Reef, Coral Sea

Emydocephalus annulatus (332) M N.L. Cogger/Moffit

Laticauda laticauda (350) N.L. Australian Museum
L. colubrina (184) M Tonga, Pacific Ocean

Australian Elapids

Acanthophis antarcticus (16) N.L. (177) F South Australia, (206) Boxer Is., N.A.
A. laevis N.L. "New Guinea" Dallas Zoo
A. praelongus (104) F Conon Hill, Arnhem Land N.T. (285) Groote Eylandt, N.T.
A. pyrrhus (124) Sand Fire Flats, W.A. (207) F " " " (307) F N.L. Naylor
A. sps. (275) F Barkly Tableland, N.T. (340) F " " "
A. sps X A. antarcticus (276) F Adelaide River, N.T.

(85) F Kangaroo Is. S.A. (137) M " (86) M " " (170) M Arroral Valley, A.C.T.
(120) F " " (272) F " (310) F Brisbane, Qld. (329) F " "

Cacophis kreffti (312) M 50 Km W. Wauchape, N.S.W.
C. aquamulosus (155) Springbrook, Qld. (190) M Sydney, N.S.W. (272) " " (310) F Brisbane, Qld. (329) " "

(182) F " (341) N.L. Shine (82) M " (321) Black Mt. Rd. via Armidale N.S.W.

C. pallidiceps (JJB 14618) F, (JJB 14619) F, (JJB 14614), (JJB 14849)
Specimens Examined Continued

Australian Elapids (cont)

Demansia atra (105) M Normanton, Qld., (122) F Conon Hill, Arnhemland, N.T.
D. olivacea (328) F Mitchell Plateau, Kimberlys, W.A., (JJJB 14642) M
D. papuensis (240) Darwin, N.T.
D. psammophis (29) Hornsby, Sydney, N.S.W., (70) Spring Cr., S. of Willmington, S.A.
(78) N.L. (323) M Mullumbimby, N.S.W.
(254) F " "
(335) F " "
(338) M
D. sps (221) Cammoweal, Qld.
D. torquata (297) M 10Km W. Acibie Beach, Qld.
Denisonia devisii (59) F Macquarie Marshs, N.S.W. (246) M Mooney, Qld.
(192) " " " (258) F " "
(295) M Narrabri, N.S.W.
D. maculata M (JJJB)
D. punctata (102) F Kurunurra, N.T.
(107) M Timber Creek, N.T. +1 (JJJB)
Drysdalia coronata (106) Esperence, W.A. + 3 same local
(235) " "
(239) "
(342) Harvey, W.A.
D. coronoides (48) N.L. (56) M trouser's Point Rd., Flinder's Is., Tas.
(69) M Flinder's Is. Tas.
(135) M S.E. Coast N.S.W. (293) M 20K E. Armidale, N.S.W.
(318) Coree Flats, A.C.T.
D. mastersi (270) F 2.75KM. N.N.W. Chinaman Well, Vic.
D. rhodogaster (26) Burrumgui Nature Res., N.S.W.
(322) F Kiola, S.E. coast N.S.W.
(324) F embryo of 322
(325) M embryo of 322
(343) Blue Mts. N.S.W.

Echiopsis curta (165) M N.L. W.A. +3

Elapognathus minor (334) F Walpole, W.A. +1 N.L.

Furina diadema (101) N.L. (119) Macquarie Marshes
(112) Townsville, Qld. (154) M 6 km N.W. of Moolawatana H.S. S.A.
(200) Burke, N.S.W.
F. ornata (131) Townsville, Qld., (JJJB 14851 and others)

Hemiaspis signata (5) Harrington Dump, N.S.W. (163) Sydney, N.S.W.
(294) Armidale, N.S.W.
(339) F Mullumbimby, N.S.W.+2

H. damelii (27) Macquarie Marshs, N.S.W.
(38) F " " "
(61) F " " "
(62) " " "
(158) F " " "
(128) M " " "
(130) F " " "
(133) F Dalby, Qld.

Hoplocephalus bitorquatus (269) F Springbrook, Qld., (JJJB 14802 F, 14804 M)
H. stephensi (196) M Springbrook, Qld.
(303) F N.L. Edwards

Neelaps bimaculata (315) M locality 47 Australian Museum
Notechis ater ater (58) P Spring Cr. S. of Willmington S.A.
(194) " " "
(197) F " " "
(251) F " " "
(306) N.L. Australian Reptile Park/Worrell
Specimens Examined Continued

**Australian Elapids (cont')**

*Notechis ater humphreysi* (15) N.L. Cann

(203) King Is., Tas.

*N. a. niger* (53) M Kangaroo Is. S.A.

(54) " "

(76) " "

*N. a. serventyi* (72) M Flinders Is. Tas. (99) Chappell Is., Tas.

(75) M " "

(87) M " "

(88) M " "

(89) M " "

(90) F " "

*N. scutatus* (10) M Bungendore, N.S.W. (31) F S. Lake George, near Bungendore

(24) M 4 km. N. Collector N.S.W. (93) M "Victoria"

(40) M " "

(41) M " "

(169) M " "

(172) F " "

(202) M " "

*N. s. occidentalis* (210) M Perth, W.A.

(211) F " "

(234) " "

(250) F " "

(257) F " "

(262) F " "

(265) F " "

(219) M Isrealite Bay, W.A.

*Oxyuranus scutellatus* (13) N.L. Toranga Park Zoo

(302) F " "

(166) F Cooktown, Qld. (JJB 14814)

*Q. (P) microlepidota* (177) M Moorney Plains, Qld.

(351) M N.L. Australian Reptile Park/ Worrell

*Pseudechis australis* (14) N.L. Toranga Park Zoo

(112) Cuddapan Air Strip, Qld.

(217) F Doongan Sta., Kimberlys, W.A.

(252) N.L. Charles (268) N.L. Shine (344) N.L. Gillam

(283) Alice Springs, N.T.

(292) Mooney, Qld.

*P. butleri* (205) F Yalgoo, W.A.

(236) F " " + 1 M

*P. guttata* (4) F Oakey, Qld. (44) embryo N.L. Cann

(204) F " " (22) F N.L. Cann

(267) F " " (45) F embryo N.L.

*P. porphyriacus* (46) F Casurina Sands, A.C.T.

(73) M N.L. (125) F N.L. Cann

(162) A.C.T. (176) F Pine Is., A.C.T.

(237) F Tianjarra Falls, N.S.W. (JJB 14598) F

(317) M 'Adnamira', Dog Trap Rd. N.S.W. +2

*Pseudonaja affinis* (212) Esperance, W.A.

(213) " "

(256) " "

(299) M " "

*P. a. tanneri* (227) M Boxer Is. W.A.

(288) F " "

(229) M " "

(216) M " "

(218) M " "

(220) M " "
Specimens Examined Continued

Australian Elapids (cont’)

Pseudonaja a. X P. nuchalis? (230) M Nulabor

P. guttata (151) M N.L., (152) N.L., (174) F N.L.
(247) M Windorah, Qld., (JJB 14774)

P. ingrani (280) F Barkly Tablelands, N.T.

P. modesta (241) N.L. (148) M Malboom out station, Mulgaathering Sta. S.A.
(JJB 14772) F

P. textilis (20) Canberra, A.C.T. (157) F Billitane, S.A.
(43) M “ “ (161) N.L.
(92) F “ “ (35) Macquarie Marshs, N.S.W.
(93) F “ “ (37) M Monto, Qld.
(159) “ “ (81) M Nowra, N.S.W.
(173) M “ “ (274) M Glen Helen Sta. N.T.
(311) Barrenjuk Dam, N.S.W. +1 Bedourie, Qld.

P. nuchalis (28) F 20Km N.W. Coober Pedy, S.A.
(226) M Ravensthorpe, W.A.
(238) M Mt. Lyndhurst, S.A.
(241) Leeman, W.A.
(273) Greenhead, W.A.

P. sps 1 “Darwin type” (244) F Darwin
(255) “ +2

P. sps 2 “Black-headed morph” (254) F Barkly Tablelands, N.T. +1

Rhinoplocephalus bicolor (208) M Many Peaks, W.A.
(209) F 15 Km E. Albany, W.A.
(224) M “ “
(225) M “ “
(231) M “ “

Simoselaps australis (25) 48 Km W. Mabel Creek H.S., S.A.

S. bertholdi (233) Perth, W.A. (1) M 10 Km W. Melba Cr. Hs. S.A.
(243) F “ “ (7) “ “
(319) F near Northcape Lodge, Exmouth, W.A.
(19) N.L.
(3) M 10 Km W. Melba Cr. Hs. S.A.

S. littoralis (320) M Australian Museum

S. warro (245) F Edward River, Qld.

(103) M Cuddapan Air Strip, Qld., (260) M Barkly Tablelands, N.T.
(327) M 10 Km N. Lake Narau, N.S.W.
(296) F Yarrabee Hs. N.W. of Wanaaria, N.S.W. +4

Tropidechis carinatus (6) M Mt. Nengo, Qld. (34) Mt. Glorious, Qld.

(84) F “ “
(193) F “ “
(309) F “ “
(263) F “ “

Unechis carpentariae (78) M Cooley Dam, (74) F Toowoomba, Qld
(118) F Mullumbimby ?
(199) F Oakey, Qld.
(266) Townsville, Qld.
(281) F “ “

U. dwoeryi (77) F Inglewood, Qld. (127) Kelly’s Flat, N.S.W.
(168) Wee Jasper, N.S.W. (277) Armidale, N.S.W.
(287) M Warrane St. via Armidale N.S.W.
(117) F Mullumbimby, N.S.W.
(271) M Townsville, Qld. (314) Tamworth, N.S.W.

U. flagellum (180) Skeleton Creek, Vic.
(183) M “ “
(186) M “ “

Specimens Examined Continued

Australian Elapids (cont')

**Unechis gouldii** (100) Chidlow, W.A.,
(289) Mt. Helena, W.A.
(349) Wubin area, W.A.
**U. monachus** (348) N.L. W.A. King, + 2 N.L. King
**U. nigriceps** (brevicauda) (232) Ravensthorpe, W.A.
(223) N.L. S.A.

**U. nigrostriata** (126) Townsville, Qld.

**Vermicella annulata** (12) N.L. N.S.W.
(21) Uki, N.S.W.
(156) Springbrook, N.S.W.
(253) F " "
(259) F " "
(290) Bukkulla, 25 mi. from Inverell, N.S.W.

Boids and Colubrids

**Typhlina bramina** (64) Darwin, N.T.
(65) " "
(66) " "
(67) " "
(96) " "
(97) " 

**T. sps** 98 N.L. Baverstock

**Acrochordus arafura** (129) Burketown Crossing near Baraloola, N.T.

**Amphiesma mauri** (32) Rainbow Bay, Qld. (18) N.L.
(47) F Tin Can Bay, Qld.
(116) M Besey Springs, Mac Arthur River, N.T.

**Boiga irregularis** (326) M Mullumbimby, N.S.W.
**Dendrelaphis punctulatus** (330) F Mullumbimby, N.S.W.
**Acrantophis dumereli** F San Antonio Zoo, F Dallas Zoo
**A. madagascarensis** (357) F Dallas Zoo

**Aspidites melanocephalus** (51) F 5 Km W of Canooweal, N.T. +4

**Candoia bibroni** (142) N.L. Dallas Zoo
**C. paulsoni** (147) N.L. San Antonio Zoo

**Chondropython viridis** (150) N.L. San Antonio Zoo

**Liasis childreni** (110) Kununurra, N.T.
(111) 40 Km E. Halls Creek W.A.
(115) F N.L.
(121) M "mica" turn off on Dajarra Rd. S. Mt. Isa, Qld.

**L. fuscus** (mackloti) (123) Cannon Hill, Arnhemland, N.T.
F 16 Km upstream from Daly R. Police Station, N.T.

**L. albertisii** (179) N.L. Melbourne Zoo
**L. olivaceus** (113) M Jim Jim, N.T.

**Morelia spilotes** (42) N.L. Cann (49) F Kenthurst, Sydney, N.S.W.
(50) M 15 Km W Wyong, N.S.W.
(17) "western N.S.W."

**Python ornellensis** (146) F N.L. Australian Museum
**P. reticulatus** (185) F captive born, Toranga Zoo
2.2 Methods

Chromosome preparations were obtained in the form of air-dried slides from cell suspensions of a variety of tissues following the treatments described below. With the exception of testicular and ovarian preparations, all other cell types were pretreated with colchicine. This, however, was reduced both in concentration and duration, compared with earlier studies (Baker et al. 1971, 1972; Mengden and Stock, 1980) giving a higher percentage of metaphase and prometaphase cells with chromosomes exhibiting close sister chromatid apposition. The use of 0.075M KCl for hypotonic treatment in preference to the more conventional 0.9% Na citrate, was also judged to promote sister chromatid apposition and this greatly facilitates chromosome banding. Most species were analysed using more than one of the techniques listed below.

2.2.1 Peripheral Blood

The technique used to obtain chromosome material from peripheral blood was similar to that described by Baker et al. (1971). Animals were injected intraperitoneally (IP) with Bacto Phytohaemagglutinin (PHA) at dilutions prescribed by Baker et al. (1971). They were then placed in plastic containers and their ambient temperature raised to 30°C. An additional injection of PHA was given 24 hours later and a further 24 hrs after this second injection, the animal was either injected intraperitoneally with 0.05 to 0.3 CC of 0.025% colchicine or blood was drawn from the caudal vein and incubated in vitro in 5 ml of a balanced salt solution containing 0.1 ml colchicine
solution. Cells, either in vivo or in vitro, were exposed to colchicine for 1.5 to 2 hrs. In the in vitro samples blood was then taken up by a heparinized syringe or capillary tube (see Branch, 1978) and subsequently centrifuged at 400 rpm for 15 min. to separate plasma, leucocytes and erythrocytes. Plasma and leucocytes were suspended in 0.075M KCl for 20 min then fixed in 3 changes of a 3:1 methanol/acetic acid mixture following centrifugation at 1500 rpm for 5 min. prior to each change of supernatant. Slides were prepared by dropping cell suspensions on wet slides and air drying.

### 2.2.2 Blood Culture

In vitro leucocyte culture proved to be both economical and time efficient and provided excellent material for banding analysis. Blood was taken from the caudal vein with a heparinized syringe. Five to ten drops of whole blood were incubated in 5 ml total volume of Hams F-10 media supplemented with 15% Foetal Calf Serum, 3 drops of PHA and 1 drop of heparin. The blood was incubated at 30°C for 72 to 86 hrs. Two hours prior to harvest, 5 drops of 0.025% colchicine were added as an arresting agent. Harvest procedure was identical to that described above. All media was removed prior to hypotonic treatment.

### 2.2.3 Tissue Culture

Monolayer fibroblast cultures were derived from primary cultures of heart, lung and skin biopsies following techniques
previously described (Stock and Mengden, 1975; Mengden and Stock, 1980). Though the procedure provides excellent material for banding analysis, it is both costly and time consuming. The number of specimens that can be analysed is thus reduced and its value in an extensive survey or population study is limited.

2.2.4 Intestinal Epithelium Preparations

A limited number of specimens were analysed using intestinal epithelium cells. This technique has been used extensively on both urodele (Kezer and Sessions, 1979; Schmid, et al. 1979 and McGregor and Sherwood, 1979) and anuran amphibians (De Leon, 1970; King, 1980) as well as geckoes (King and Rofe, 1976). The technique involves removal of a section of intestine from an animal pretreated with colchicine. The intestine is opened and the epithelium gently scraped into a hypotonic solution. The procedure then follows standard hypotonic and fixative steps. Prolonged fixation (12 or 24 hrs) often helps to spread the chromosome even after hypotonic treatment (King, pers. commun.). Because of the high instance of incomplete cells, and hence incorrect chromosome counts, when applied to the elapid material and the inability of other authors to G-band intestinal epithelial cells it was not used extensively in this study.
2.2.5 Gonadal Preparations

Both male and female gonadal tissues provide suitable mitotic material when animals are pretreated with colchicine. Gonadal tissues were excised and finely minced, then drawn through a 19g needle to homogenize cells. Hypotonic, fixative and slide treatments follow those described in section 2.2.1. Meiotic preparations can be obtained in a similar fashion from males that have not been treated with colchicine. Becak and Becak (1981) have used a similar technique to obtain meiotic preparations from female snakes.

2.2.6 Postmortem Chromosome Preparations

It is possible to obtain gross karyotypic preparations from animals that have been dead for up to 12 hours. Viable tissue cultures can be established from subcutaneous fibroblasts twelve or more hours after death (Mengden, unpublished). Additionally, preparations with morphology suitable for banding analysis can be obtained from bone marrow flushes and gross preparations from intestinal epithelium postmortem after 1 hr in vitro exposure to 0.001% colchicine in mammals, birds and reptiles. These techniques have been applied during the course of this study in instances when damaged animals were obtained which died prior to treatment and even to road-killed specimens.
2.3 Banding Techniques

The chromosome banding techniques described in this section were all developed in human cytogenetic laboratories over the past decade. Despite the advantages of these techniques over gross karyotypic data alone (see section 1.2) they have been applied to reptilian material in only relatively few instances. This may be because these techniques need to be modified slightly for application to non-mammalian tissues which are generally considered more difficult to band.

2.3.1 C-bands

The two C-banding techniques utilized differ in the alkaline base used. The NaOH technique was similar to that reported for bird material by Stephos and Arrighi (1971) and subsequently modified by Mengden and Stock (1975). This, however, was found to be too harsh a treatment due to the strong alkaline quality of the NaOH solution and the prolonged exposure (12-18 hrs) to the 2 x SSC solution. Under these circumstances some of the smaller interstitial C-band blocks may well be destroyed by such radical treatment.

A C-banding technique utilizing hot BaOH, similar to the one described by King (1980), was found to be preferable to the NaOH technique described above. Slides were 'aged' by exposure to room temperature for one to ten days or by placing overnight in an incubator at 55°C. They were pretreated with 0.2N HCl for 20 min. then rinsed thoroughly in distilled H2O. A saturated solution of BaOH was stirred for 1 hr. and allowed to stand to remove carbonate by crystalization. This
purified solution was placed in a water bath at 45°C. Slides were exposed to BaOH at 45°C for 40 to 120 sec. then dipped briefly in 0.2N HCl and rinsed in distilled water. They were then placed in 2 x SSC at 65°C for 60 min, again rinsed in distilled water and stained with 10% Giemsa in pH 6.8 PO₄ buffer. When chromosome preparations appeared darkly stained, as in gross karyotypes, they were retreated by brief exposure to BaOH and a 30 min. incubation in SSC.

2.3.2 G-Bands

A variety of G-band techniques were applied to chromosome preparations used in this study, however only the most reliable and repeatable of these is described in detail here.

Slides were aged at room temperature for 3 days to several weeks. This 'ageing' process is thought to make cells more resistant to the trypsin treatment and thus avoid over treating. An attempt to standardize this process was made by placing air dried slides in an incubator overnight (12-14 hrs) at 50°C or by exposing them to a 20% by vol. solution of H₂O₂ for 20 min followed by rinsing in distilled water. The trypsin solution contained 1% Trypsin (Difco) dissolved in Hanks basal salt solution, without Ca++ and Mg++, and supplemented with Versene. The treatment time was 8 to 30 sec. Slides were immediately rinsed in Hanks BSS, 70% ETOH, 95% ETOH then air dried. They were then stained in a 5% Giemsa solution for 5-15 min.

An alternative G-band technique, similar to that described by Wang and Fedoroff (1972), involved the use of a dilute trypsin solution, 0.025-0.1% in Hanks basal salt solution at
30°C. This increased treatment time to 1-5 min and thus reduced the incidence of overtreating.

Perhaps the most critical factor in G-banding is the morphology of the chromosomes to be banded. Close sister chromatid apposition is essential for clear results and treatments to ensure this are mentioned in section 2.2 above. While contracted metaphase cells will G-band, the more elongate pro-metaphase chromosomes show finer differentiation of bands along the chromosomes. The elongated pro-metaphase morphology with sister chromatids in close apposition is difficult to achieve from gut epithelia preparations and this may well be the factor that has prevented some workers successfully G-banding anuran materials.

2.3.3 N-bands

A variety of techniques are available for the characterization of nucleolar organiser regions (NOR's) in metaphase chromosomes. The value of NOR morphology, location and variation in phylogenetic studies has been suggested above. There is much debate concerning the relative accuracy of these banding techniques in characterizing the presence and/or activity of ribosomal genes and their products. By the application of several techniques in this study I hope to not only define with some confidence the location of NOR's in the species studied but also to analyse the action of these techniques upon reptilian cells. In a recent study on NOR's
of the grasshopper *Warramaba virgo* and its relatives, White et al. (1982) have found N-banding to be in total agreement with the localization of both the 18S and 26S RNA and the 5S RNA genes as characterised by *in situ* hybridization of two separate *Drosophila* probes for those genes. As will be apparent from the present study, such a close correlation may not obtain in other groups of animals.

The N-band technique used here is identical to that of White et al. (1982) which follows Gerlach (1977). Slides were standardized by placing on a "hot plate" at 40-50°C for 30 min. They were then treated for 5 min. at 95°C in 1M NaH$_2$PO$_4$ at pH 4.2 followed by distilled H$_2$O and then stained for 10 min in 10% Giemsa in PO$_4$ buffer (see above).

2.3.4 Ag Staining

Again a variety of Ag staining techniques were utilized, including that of Goodpasture and Bloom (1975) and 50% aqueous AgNO$_3$ + .2% formic acid in a 7:1 ratio incubated at 50°C, as described by Olert (1979) and used by White et al. (1982).

2.4 Fluorescence

Some workers have reported a correlation between the binding of certain fluorochromes and the underlying base composition of a chromosome region. Whatever the cause of the staining specificity of these fluorescence banding techniques they provide additional markers and, in some cases, allow a more precise definition of categories of heterochromatin
comprising what would otherwise appear as homogenous C-positive blocks (see sex chromosome delineation, Chapter 4). Recently developed counter-staining with competitive fluorochromes has enhanced the resolution of these banding techniques.

2.4.1 Q-bands

Slides of various ages where stained for Q-bands following the procedure used by Stocker et al. (1978).

2.4.2 Tri Staining Technique for R and DA-DAPI Bands

This technique is discussed in detail by Schweizer (1980, 1981) and involves staining for 1 hr with chromomycin A3 (Sigma) 0.5 mg/ml dissolved in 50% McIlvaine's, rinsing with distilled water, staining with distamycin A (Sigma), 0.1 mg in 1 ml of McIlvaine's for 15 min., as a counterstain and finally staining with DAPI, 0.6 µg/ml in McIlvaine's buffer, for 20 min before a final rinse and mounting in a 1:1 glycerol/McIlvain mixture. In theory this technique visualizes GC rich bands when viewed with barrier filters allowing a 435 nm exposure and AT-rich DAPI bands at 360 nm. Because the cell is stained with chromomycin, counterstained with distamycin A, and then with DAPI, both types of bands can be observed in the same cell by simply changing the barrier filter.
2.4.3 **DAPI Bands**

In addition to the technique of Schweitzer (1981) described above, DAPI bands were also obtained by the following technique involving counterstaining with Actinomycin-D. The slide was stained with DAPI, 0.6 µg/ml in McIlvaine's buffer, rinsed and stained with actinomycin-D, 0.3 mg/ml in 10 mM phosphate buffer pH 7, before a final rinse and subsequent mounting in McIlvaine's buffer.

Color photos were taken with a Zeiss fluorescence microscope using Kodak Ektachrome (Professional) ASA 160 E6 process. Black and white photos were made with Kodak Copex fast film.

2.5 **In situ Hybridization**

The in situ work reported here was performed on chromosome preparations of elapid material provided by myself but carried out by Dr. L. Singh (Department of Genetics, University of Edinburgh, Scotland) in the case of the Bk minor satellite hybridization and by Dr. R. Honeycutt, of my own Department, in the case of the 18S + 28S rRNA Drosophila probe.

2.5.1 **BK Minor Satellite Hybridization**

Singh et al. (1976, 1980) have reported that the Sat III DNA of the colubrid *Elaphe radiata* and the BK minor satellite of the elapid *Bungarus* are indistinguishable in composition and are both sex specific for the W chromosomes of female...
snakes. Dr. Singh has kindly labelled a few elapid preparations provided by me with the BK minor satellite. This data will be referred to in Chapter 4, as it relates to the phenomenon of linear differentiation of heterochromatin on the W chromosome of some vertebrates.

2.5.2 18 and 28S rRNA *Drosophila* Probe Hybridization

In order to determine the precise location of the 18 + 28S ribosomal genes, *in situ* hybridization was carried out utilizing a probe for those genes derived from *Drosophila* material. This technique was performed by Dr. R. Honeycutt on selected elapid material provided by me. As a control to compare the sensitivity of the *Drosophila* probe to vertebrate ribosomal genes the technique was also applied to the anuran *Xenopus* in which the site of 18 and 28S rRNA production is well documented. To gauge the reactivity of each experimental run, and to determine the optimal exposure time, the probe was applied to preparations from the anuran *Litoria* which are known to possess very large NOR's (King, 1981). There was a good correlation between the *Drosophila* probe and 18 and 28S rRNA production sites in *Xenopus*. Unfortunately, no 5s probe was available at the time of this study.

2.6 Nomenclature

Fig. 2.1 illustrates terms used to describe gross karyotypic appearance and C-band location.
Figure 2.1 The terms used to describe centromere position and C-band location are illustrated here. Likewise, synon­nomies which may appear in this and other works appear below each illustration.
Nomenclature

Centromere Position

Median
Metacentric

Submedian
Submetacentric

Subterminal
Subtelocentric or Subacrocentric

Terminal
Telocentric
Acrocentric

C band Location

Centric

Procentric
Proximal

Interstitial

Telomeric
Distal
Comparative Chromosome Morphology and Banding Patterns in the Elapid Snakes

3.1 Characterization of Karyomorph Groups

In an attempt to rationalise the large number of taxa examined in this thesis the chromosome data has been grouped into a series of karyomorphs on the basis of shared similarities between specific karyotypes. A classical description is then provided for representative species within each karyomorph, both with regard to gross karyotypic morphology and C, G and N-banding patterns, silver staining and fluorescence.

Where G-banding data is not available the assignment of a particular species to a karyomorph must be considered tentative. Additional banding and fluorescence data are provided in subsequent Chapters and the major comparisons within and between groups are reserved for the Discussion (Chapter 6). The groupings referred to in this Chapter are thus largely a descriptive convenience and should not necessarily be taken to denote phylogenetic affinities. Finally, while the G-band data illustrated here is from typically condensed metaphase preparations, a greater variety of G-band preparations, including prometaphase cells, are used to determine the G-band sequence homologies discussed in Chapter 6.
3.2 The Naja Karyomorph

Naja is the only terrestrial elapid genus not restricted to a single continent. Gross karyotypic data has been published for five taxa in the genus (see Table 1.2, Chapter 1) and all possess a diploid number of 38. Though there has been some disagreement as to the number of macro and microchromosomes this is simply a matter of semantics. All taxa possess five distinctively larger pairs of macrochromosomes. Some authors have recognized 3 more pairs of "intermediate" macrochromosomes (Singh, 1972; De Smet, 1978) but data presented here for the same species clearly shows a continuous size gradation from pair 6 to pair 19 in all species and all these elements are therefore considered to represent microchromosomes (Fig. 3.1). C-band data for four taxa have been examined in this thesis, including N. nigricollis, N. haje and N. n. kaouthia, for which gross karyotypic data has been previously published (De Smet 1978; Singh 1972), and N. melanoleuca for which no data has been previously available. Additionally G-band data is presented for N. haje and N. n. kaouthia. The African species, N. nigricollis N. haje and N. melanoleuca appear to have minute short arms on the 2nd and 14th largest pairs of chromosomes in comparison to the larger short arms on these chromosomes in the Asian subspecies of N. naja. C-banding indicates both centric and telomeric heterochromatin on the 5 pairs of large macrochromosomes (Fig. 3.1). Such telomeric heterochromatin is not obvious in N. naja. Most of the larger microchromosomes appear biarmed in Naja and possess large amounts of heterochromatin.
Figure 3.1. Gross karyotypic (upper) and C-banded preparations (lower) of female *Naja melanoleuca* (2n = 38, 10M/28m). The sex chromosome pair is the fifth largest pair of macro elements. Note telomeric heterochromatin on all macro elements as well as interstitial C-bands on pairs 1, 4 and the Z. Refer to text, sections 3.2, 4.3, 6.62 and Figure 4.1.
Singh (1972) suggested that the sex pair in *N. n. kaouthia* is strongly heteromorphic in most specimens examined and Desmet (1978) has identified pair 5 as the heteromorphic pair in *N. nigricollis* (see Fig. 3.2) and the same applies to *N. n. kaouthia* (Fig. 3.3). Singh *et al.* (1980) state that, in addition to the centromeric regions of the autosomes, the W chromosomes of *N. n. oxiana* and *N. n. naja* are completely heterochromatic. In contrast, the C-band data presented here indicates that the W chromosomes of all four taxa examined are C-band positive but show linear differentiation of lighter and darker C-band regions (see Chapter 4). In *N. haje* the darker C-band region of the W appears DAPI positive in fluorescence, further attesting to the heterogeneous composition of this sex chromosome (Fig. 4.4).

No prominent secondary constrictions were observed in any of the gross karyotypic preparations. Nor have they been reported previously in the literature. Silver staining, however, suggests that the nucleolar organizer region (NOR) is located on a median size microchromosome which is sub-metacentric in gross morphology (Fig. 6.1C). The *in situ* hybridization studies labelling for 18s + 28s rRNA confirm this observation. The homologues possessing NOR sites are frequently seen to associate in mitotic metaphase in a wide variety of species where these regions are exclusively located on the microchromosomes (Fig. 6.1A, E, F).

In conclusion, it appears that the species of the genus *Naja* form a cohesive and distinct karyomorph group with minor differences in C-band character between African and Asian species. In terms of banding details all of them possess species-specific W chromosomes. *N. n. oxiana* is the most
Figure 3.2. Gross karyotypic and C-band data for *Naja nigricollis* (2n = 38, 10M/28m). Lower - gross karyotype for *Naja haje* (2n = 38, 10M/28 m). See text sections 3.2 and 6.6.2, also fluorescence Figure 4.4 and G-band Figure 6.8.
Figure 3.3. (A) *Naja naja kaouthia* (2n = 38, 10M/28m), gross karyotype and C-band preparation of the W sex chromosome. Note submetacentric pair 2 possesses short arms. (B) C-band preparation of *N. n. polyocellata*. Unlike other *Naja* this species possesses a W larger than the Z. Note also that both species illustrated here show taxon-specific linear differentiation of lighter and darker C-band regions along the W chromosome.
distinctive subspecies of *N. naja* with a submedian pair 4 as opposed to the subterminal condition seen in other *Naja*. The lack of large whole arm C-band regions in this and other Asian *Naja* suggests that pericentric inversion, rather than heterochromatic addition, is responsible for short arm differences between species inhabiting the two continents.

3.3 African Elapids Exclusive of Naja

3.3.1 The Chromosomes of Hemachatus haemachatus

The chromosomes of three male specimens of the monotypic genus *Hemachatus* have been analysed using a wide variety of staining techniques. This genus is characterised by a diploid number of 42 with 16 macro chromosomes and 26 micro chromosomes. Pairs 1 and 3 are metacentric, pair 2 is submetacentric and the remainder of the macro chromosomes are acrocentric (Fig. 3.4). Only one pair of micro chromosomes appears distinctly biarmed.

Due to the lack of availability of female specimens the Z chromosome cannot be accurately identified. The fourth largest pair of chromosomes is often the sex pair in snakes. In *H. haemachatus*, however, the fourth pair is acrocentric, a condition not previously reported for the Z chromosome of any snake species. Pair 3 possesses a gross morphology and C-band character more typical of the Z chromosomes observed in other elapid species. Additionally it exhibits a DAPI negative fluorescence in juxtaposition to a telomeric C-block which is
Figure 3.4. *Hemachatus haemachatus* (2n = 42, 16M/26n) gross karyotypic (upper), C-band (middle) and G-band (lower) data. Note prominent centromeric C-block on all autosomal macrochromosomes and telomeric C-bands on the short arm of the Z. Some of these heterochromatic C-bands appear DAPI positive in fluorescence (see Fig. 5.6). G-band data is compared with other African and Asian elapid species in Fig. 6.8. See text sections 3.3.1 and 6.6.2.
unique among the autosomes. A Robertsonian rearrangement of the typically larger metacentric pair 3, equivalent to that believed to have occurred in *Pseudechis australis* (see Section 3.5.6), might account for this apparent anomaly. Though G-band comparisons of *Hemachatus* and other elapids suggest possible homologies between certain of the bands of the Z chromosome, the absence of female specimens and the monotypic nature of this genus makes accurate sex chromosome identification impossible at this time.

The C-band positive areas of the chromosomes of *Hemachatus* are restricted to the centromeric regions of all autosomes except for pair 3 which possesses a faint centromeric C-block and a more prominent telomeric C-band on one arm. All other macrochromosomes possess quite large C-blocks in the centromeric regions. These are most developed on the acrocentric pairs, 4-8. All microchromosomes also exhibit centromeric C-bands and one pair appears almost completely C-band positive. The large blocks associated with the centromeres are also densely G-band positive on all the macrochromosomes except pair 3 (Fig. 3.4) and are intensely DAPI fluorescent on pairs 1 and 2 (Fig. 5.6). Such an intense G-band reaction in a C-band region is exceptional though it has been seen to a lesser extent in *Ophiophagus* (see Fig. 3.9) and is associated with the presence of secondary constrictions in *Acanthophis* (see Fig. 5.4). In this respect *Boulengerina* (Fig. 3.5) resembles *Naja* rather than *Hemachatus*.

No secondary constriction is apparent in any of the *Hemachatus* preparations; however both the *in situ* hybridization of the 18s + 28s rRNA probe and the silver stain unequivocally demonstrates the NOR to be located on one of the
Figure 3.5. *Boulengerina annulata* (2n = 38, 10M/28m) most closely resembles *Naja* in gross karyotype. In the specimen illustrated here there is a size heteromorphism of pair 2. C-banding (B) demonstrates this to be due to an addition of heterochromatin in the procentric region of one of the homologues. G-banding (C) shows the procentric heterochromatin to be G-band negative.
microchromosome elements (Figs. 6.1 and 6.2). Due to the minute size of these chromosomes the exact element cannot be identified with certainty though the C-band positive microchromosome pair is a likely candidate. As in Naja and Ophiophagus, homologous micro-elements possessing NORs are often associated with one another even at metaphase (Fig. 6.1A).

3.3.2 The Aspidelaps Karyomorph

Some information on the gross chromosome morphology of a male Aspidelaps scutatus is available in the PhD thesis of Fischman (1968), however this data remains unpublished. I have analysed banding data from females of both species currently recognised within the genus Aspidelaps and found them to possess a diploid number of 40 with 16 macrochromosomes and 24 microchromosomes though there is a gradual decrease in size only from pair 7 to 10 (Fig. 3.6A). The four largest pairs of chromosomes appear conserved, as is the case with many elapid species. Pairs 1 and 3 are metacentric, while pair 2 is submetacentric and pair 4, the Z, is only slightly submetacentric. Pairs 5 to 7 are acrocentric in A. scutatus, pair 8 has minute short arms, while pairs 9, 10, 12 and 14 have distinct short arms even though they belong to the microchromosome class. By contrast, in A. lubricus pair 5 possesses distinct short arms, whereas these are not as obvious on pair 8 as in other species of this genus.

The W sex chromosomes of the two species of Aspidelaps differ radically. In gross morphology the W of A. lubricus is
Figure 3.6. Gross karyotypic and C-banding data on the species of the African elapid genus *Aspidelaps*. (A) - Gross karyotype of *Aspidelaps scutatus* (2n = 40, 16M/24); (B) - C-band data on *A. scutatus*; (C) - C-banded sex chromosomes of *A. lubricus* and *A. scutatus*. Note differences in gross morphology and C-band character of the W. *A. scutatus* also lacks the C-band positive centromeric region of the Z; (D) - C-band data for *A. lubricus*. Note biarmed nature of pair 5. For further discussion, see text sections 3.3.2 and 6.6.2.
slightly submetacentric and larger than the Z. In *A. scutatus* the W is acrocentric and larger than the Z. C-banding data shows additional species differences. While the Z chromosome of both species possesses a telomeric C-band on the long arm, the W chromosome displays species-specific linear patterns of lighter and darker C positive regions (Figs. 3.6C and 4.1). In *Aspidelaps scutatus* the short arms of pairs 8 and 9 appear completely C-band positive (Fig. 3.6D). This is not the case for the short arms of pair 5 in *A. lubricus* (Fig. 3.6D). The latter species also possesses more prominent centromeric C-bands on pairs 2 and 3 than does *A. scutatus*. No secondary constriction or nucleolar organizer region has been assuredly identified in either species.

In conclusion, the two species of *Aspidelaps* form a cohesive and distinctive karyomorph among the African elapid species though species-specific differences in sex chromosomes and slight differences in gross karyotypes can be attributed to both heterochromatic addition and pericentric inversion.

### 3.3.3 The Chromosomes of Walterinnesia

In examining a single male specimen of the rare Desert Cobra, *Walterinnesia aegyptia*, I found that it too possesses a unique karyotype among African elapids with a diploid number of 44 including 22 macro and 22 microchromosomes. The typically metacentric pair 1 appears to be replaced by two acrocentric elements, pairs 2 and 3, while the submetacentric element usually defined for pair 2 is conserved but is now the largest element in the genome, pair 1. Pair 4 is submetacentric and
may represent the Z. Pairs 5, 7, 9, 10, 11 are acrocentric macrochromosomes while pairs 6 and 8 possess minute short arms. Microchromosome pairs 12 through 22 appear largely acrocentric.

There exist large and prominent C-band blocks in the centromeric regions of acrocentric chromosomes pairs 2 and 3 while submetacentric pair 4 and acrocentric pair 7 possess fainter centromeric C-bands (Fig. 3.7).

3.3.4 The Chromosomes of Dendroaspis

As pointed out in Chapter 1, the genus Dendroaspis displays unique cranial and venom characteristics, the analysis of which have caused some authors to suggest that the genus represent a distinct lineage among African elapids. Unfortunately, due to a paucity of specimens available from collections within Australia and the United States, and the difficulty in safely handling them, I have had access to only two of the four species in this genus, viz Dendroaspis angusticeps and D. polylepis. Of these I present gross karyotypic data for only the Black Mamba, Dendroaspis polylepis. This species exhibits a karyotype unlike that of any other African elapid studied. With a diploid number of 36, including 16 macro and 20 microchromosomes, this snake appears to conform to the "classical" karyomorph seen in many snake species (Fig. 3.8). The first three chromosome pairs are metacentric, submetacentric and metacentric respectively, suggesting that they may be conserved in comparison to other elapids. Pair 4 again represents the sex chromosome pair,
Figure 3.7. *Walterinnesia aegyptia* male (2n = 44 22M/22m). Gross karyotype (upper) and C-banded preparation (lower). Unlike many elapid species, pair 1 is submetacentric. Also note the prominent centromeric C-bands on pairs 2 and 3. See text sections 3.3.3 and 6.6.1.
Figure 3.8. The gross karyotype of *Dendroaspis polylepis*, with a diploid number of 36 composed of 16 macro and 20 microchromosomes, is unique among African elapid genera. See text sections 3.3.4 and 6.6.2.
with a submetacentric Z chromosome and a large metacentric to submetacentric W that approaches the third largest element in total length. Pair 5 is metacentric and pairs 6 through 8 are acrocentric with minute short arms, though with pair 6 possessing the largest short arms of this series. The gross morphology of the microchromosomes are especially clear in the preparation illustrated, due to its prometaphase condition. The first two microchromosome pairs are acrocentric, with obvious short arms, and are followed by three pairs of metacentric microchromosomes. Of the remaining five pairs of microchromosomes only one appears metacentric.

No obvious secondary constrictions were visible in the material. It would clearly be desirable to examine additional species of this genus to determine if G-band homologies exist between Dendroaspis and other, non-African, elapids sharing a similar gross karyotype.

3.4 The Asian Elapids

Some species of Naja described above inhabit Asia. In addition, chromosome data has been published for three species of the genus Bungarus (see Table 2, Chapter 1).

3.4.1 The Chromosomes of Ophiophagus

The King Cobra belongs to the monotypic genus Ophiophagus. I have examined two male species of Ophiophagus hannah using a wide variety of chromosome banding techniques. Here again we see a unique karyotype among
elapids. The 18 macrochromosomes and 18 microchromosomes collectively constitute a diploid count of 36 (Fig. 3.9). The first three chromosomes pairs are metacentric, submetacentric and metacentric respectively. Pair 4, which I assume to be the Z chromosome pair, is submetacentric. Pair 5 possesses obvious short arms while pair 6 is again submetacentric. Pairs 7 through 9 are acrocentric with minute short arms. At least three pairs of microchromosomes appear biarmed.

Although there are no obvious secondary constrictions in these preparations, extensive data from silver staining consistently demonstrate a silver positive region on both homologues of the largest acrocentric microchromosome pair (Figs. 6.1). These homologues are seen to associate at the presumed NOR in 80% of the mitotic metaphase cells examined (Figs. 6.1). A similar silver positive NOR has been identified by in situ hybridization using a 18s + 28s rRNA probe on the microchromosomes of Hemachatus and Naja (see Fig. 6.2). Due to the minute size of these elements it is impossible to demonstrate precise homologies between them.

C-banding demonstrates clear centromeric C-bands on macrochromosome pairs 1 through 6, with a particularly prominent centric block on pair 3 (Fig. 3.9). Pair 2 possesses an additional C-block in the procentric region of the long arm. DAPI fluorescence indicates the blocks on pairs 2 and 3 are intensely DAPI positive, suggesting that they may have a similar DNA composition which differs from the other C-band positive regions on the autosomes (Fig. 5.6). G-band preparations (Fig. 6.8) indicate a prominent G positive band associated with this satellite on both chromosomes. Indeed
Figure 3.9. The karyotype and C-banded macrochromosomes of the King Cobra *Ophiophagus hannah* (2n = 36; 18M, 18m). As only male specimens were available, identification of the sex chromosome pair is tentatively based on presumed G-band conservation with other elapids (Fig. 6.8). See text.
this also appears to be the case in the G-band data from other genera where DAPI positive C-blocks have been identified on the autosomes (see for example, Hemachatus and Acanthophis).

3.5 The Australian Elapids

3.5.1 The Notechis Karyomorph

*Notechis scutatus* is the only Australian elapid for which gross karyotypic data has been published (Shine and Bull, 1977). Subspecific variation in the C-band character of the W chromosome in this and other elapids has, however, already been demonstrated by Mengden (1981) and will be elaborated on in the following Chapter. Gorman (1981) has suggested that the gross similarity between the karyotypes of *N. scutatus* and some laticaudine sea snake species may indicate that this karyomorph is primitive for the elapid radiation. In the light of the range of karyomorphs reported here for Australian terrestrial elapids this conclusion may well prove to be blatently oversimplistic.

In gross karyotypic appearance all subspecies of *Notechis* (sensu Cogger, 1979) possess 14 macrochromosomes and 20 microchromosomes. Pairs 1, 3 and 6 are metacentric, while pair 5 and the Z chromosome are very slightly submetacentric. Pair 2 is submetacentric to subacrocentric and pair 7 is acrocentric. At least 7 pairs of microchromosomes are biarmed. There is a prominent secondary constriction on one arm of pair 1 (Fig. 3.10).
Figure 3.10. *Notechis scutatus* (2n = 34; 14M/20m). Gross karyotype (upper), C-band (middle) and G-band (lower) preparations. Note prominent telomeric and centromeric C-bands on all macroelements, as well as interstitial bands on pairs 1 and 7. Refer to text sections 3.5.1 and 6.6.1.
All autosomes and the Z possess centromeric as well as telomeric heterochromatin. Particularly large blocks of heterochromatin are seen in the telomeric region of the long arm of chromosome 2, the short arm of the Z and in the procentric region of the acrocentric pair 7. Small heterochromatic nodules are seen in the region of the secondary constriction on pair 1 and in the procentric region of the long arm of pair 2. The secondary constriction on pair 1 is Ag-positive and hybridises with a 18S and 28S rRNA probe. Differential expression of these small C-band areas may be a treatment artifact. The fluorescence data indicates clearly that the heterochromatic areas are not all of identical composition (Fig. 4.3). Likewise, the W chromosome, which appears acrocentric with only a minute short arm in all subspecies of *Notechis* (Figs. 3.11 and 3.12), possesses subspecies specific patterns of lighter and darker C-positive heterochromatin, while fluorescence data indicates that the composition of W chromosome is neither homogeneous along its length nor analogous between subspecies (Fig. 4.1B).

A very similar chromosome complement is seen in all species of the genus *Notechis* (Fig. 6.4) as well as *Austrelaps* (Fig. 3.13) and *Tropidechis* (Figs. 3.14, 4.3 and 6.4) even with regard to the details of C- and G-banding. The W chromosome again varies between species in composition though not gross morphology (Figs. 4.1 and 4.3, compared to Figs. 3.10 and 3.14) and variation can be seen in the G-band pattern of pair 7. The G-bands are presented here for species representing all three genera but are compared in detail in Chapter 6 (Fig. 6.4). It is interesting to note that Storr (in press) has
Figure 3.11. *Notechis s. occidentalis* gross karyotypic and C-band details of the macrochromosomes. Note the taxon specific banding pattern of the W sex chromosome and compare with Figure 3.10, 3.12 and 4.1. See text sections 3.5.1 and 6.6.1.
Figure 3.12. The chromosomes of *Notechis ater* niger (top) and *N. ater* ater (below) in gross and C-band preparations. Note slight differences in C-band details between these and the Figure (3.10) for *Notechis scutatus* as well as the W chromosome banding (Fig. 4.1). See text sections 3.5.1 and 6.6.1.
Figure 3.13  *Austrelaps superba* (2n = 34, 14M/20m). Note in the gross karyotypic preparation (above) of the male it is not possible to identify the Z chromosomes. In the G-banded preparation offered (below) the Z appears to be the fifth largest chromosome. This highlights the close relationship between the group 4 and group 5 species, see sections 3.5.1 and 6.6.1 for further discussion and refer to Figure 6.4 for a comparison of G-bands between these species groups.
Figure 3.14. *Tropidechis carinatus* (2n = 34, 14M/20m) gross karyotypic (upper), C-band (middle) and G-band (lower) preparations. Note the similarity between this species and *notechis* in both gross karyotype and banding details (compare with Fig. 3.10 and see also Fig. 6.4). See text sections 3.5.1 and 6.6.1.
included the species of *Austrelaps* under the genus *Notechis* on the basis of superficial morphological characters, while Morrison et al. (in press) have found a remarkable similarity between the venoms of *Notechis* and *Tropidechis* so that it appears that the species which I have included in this karyomorph share other common characteristics.

### 3.5.2 The Drysdalia Karyomorph and its Variants

All species of *Drysdalia* recognised by Cogger (1979), as well as *D. rhodogaster* subsequently redescribed by Coventry and Rawlinson (1980), have been analysed by banding in this study. They each exhibit a secondary constriction on one arm of the largest autosomal pair. With the exception of *D. coronata*, which possesses $2n = 36$ with 16 macro and 20 microchromosomes (Fig. 3.15), all other species have a diploid number of 34 with 14 macrochromosomes and 20 microchromosomes. In gross karyotypic appearance *D. coronoides*, *D. mastersi* and *D. rhodogaster* have a superficial resemblance to the *Notechis* karyomorph and a comparison of G-bands between these groups (Fig. 6.4, Chapter 6) indicates that chromosome pairs 1, 2, 3, and to a lesser extent 7, have been conserved. The G-band negative centromeric block on the acrocentric chromosome 7 of *Notechis* corresponds to the large C-band positive block reported above. Since a prominent C-band is lacking in all three *Drysdalia* species, the lack of the corresponding G negative band is to be expected.

The unique characteristic of the karyomorph of these three *Drysdalia* species is the sex chromosome pair. The W
Figure 3.15  Chromosomes of the genus Drysdalia. Gross karyotypic data from male (A) and female (B) specimens of D. coronata (2n = 36, 16M/20m).  (C) C-band data from a female D. coronata.  (D) C-band data from a female D. coronoides. Gross karyotypic data for (E) D. mastersi, (F) D. rhodogaster and (G) D. coronoides. For G-band comparisons of the Drysdalia species see Fig. 6.4. Note here that D. coronata possess the "primitive" Australian elapid karyomorph with 16 macro and 20 microchromosomes. The three smallest macro elements are acrocentric. All other Drysdalia species have a diploid number of 34 with 14 and 20 microchromosomes. Additionally the sex chromosomes are the fifth rather than fourth largest pair. The derivation of this condition and its significance is discussed in text sections 3.5.2 and 6.6.1.
chromosome is acrocentric and species-specific patterns of both
G- and C-band differentiation exist. The Z chromosome too
appears highly modified. While in most elapids, indeed in
most snake species, the Z chromosome is the 4th largest pair,
in Drysdalia it is clearly the 5th largest pair. Additionally
the Z appears to vary in morphology between species, being
subacrocentric in D. mastersi and submetacentric in D.
coronoides and D. rhodogaster, and this variation occurs
without apparent alteration of the autosomes. Careful G-band
analysis (Fig. 6.4, Chapter 6) will determine if this condition
represents an independent derivation of the Z chromosome or a
modification of the Z found in most other species.

Finally, it should be reiterated that Drysdalia coronata
is unique among the species of this genus in possessing a
diploid number of 36 (16M/20m) with the three smallest macro-
chromosomes being acrocentric. Such a configuration is also
found in a variety of unrelated genera. The C-band character
of D. coronata is seen in Fig. 3.15.

The variation within this genus may be significant for
taxonomic reasons. It will be recalled from the review in
Chapter 1, that McDowell (1968) reported an unexpected
resemblance between Drysdalia on the one hand, and Notechis,
Tropidechis and Oxyuranus on the other. Indeed he could find
no internal characters separating D. coronata and Notechis.
Storr (in press) is in the process of synonymizing Drysdalia,
Austrelaps, Elapognathus and Echiopsis under Notechis.
McDowell, however, examined only D. coronata among the
Drysdalia species and Storr has not considered Tropidechis or
Oxyuranus. The real significance of the chromosomal variation
in Drysdalia will thus be apparent only when comparisons between all these groups in question are carried out in Chapter 6.

3.5.3 The Oxyuranus-"Parademansia" Karyomorph

Oxyuranus scutellatus possesses a diploid number of 36 with 16 macro and 20 microchromosomes. Again there is a prominent secondary constriction on pair 1 and the first three pairs of autosomes appear identical, at least in gross morphology, to the equivalent chromosomes in Notechis, Drysdalia, Pseudechis and indeed in the majority of Australian terrestrial elapids. The sex chromosome pair in Oxyuranus is again the 4th largest pair of chromosomes. The Z is submetacentric as too is the W which is, however, considerably larger than the Z being the 3rd largest chromosome in the complement. It also shows linear differentiation in C-band character. Pairs 1, 3, 8 are metacentric, or very nearly so, while pairs 2, and 5 are submetacentric. Pairs 6 and 7 appear acrocentric with pair 7 displaying a prominent short-arm. Three pairs of micros are median to submedian and an additional two pairs appear to have very short arms. The others are assumed to be acrocentric (Fig. 3.16).

The most obvious feature of the C-banded karyotype of Oxyuranus scutellatus are the large C-blocks at the centromeric region of all macrochromosomes, including the Z. The centromere of most microchromosomes are also C-band positive. A fainter telomeric C block is apparent on the long arm of pair 2 in most cells. On metacentric to submetacentric pair 8 the
Figure 3.16  Chromosomes of the genus *Oxyuranus*. A and B = *O. scutellatus*, C-H = *O. (P.) microlepidota*. A, E and F are gross karyotypic preparations, B, C, D and F are C-banded preparations and H is a G-band preparation. Both species have a diploid number of 36 with 16 macro and 20 microchromosomes. *O. scutellatus* possesses larger centromeric C-bands on the five largest chromosome pairs than does *O. microlepidota*. For further discussion refer to text sections 3.5.3 and 6.6.1. G-band comparisons of this and other genera possessing a diploid number of 36 appear in Chapter 6.
centric C-block appears to extend over much of one arm (Fig. 3.16).

Only a single, male, specimen of Parademansia (O.) microlepidota was available for chromosome analysis. As I have earlier reported, in Covacevich et al. (1981) the chromosomes of P. (O) microlepidota appear almost identical in gross morphology and C-band character to O. scutellatus. Pairs 6 and 7 of P. (O) microlepidota are again acrocentric, though 7 does not exhibit as obvious a short arm as O. scutellatus (compare Figs. 3.22 and 3.23). On the basis of a suite of characters Covacevich et al. (1981) have placed Parademansia microlepidota in the genus Oxyuranus.

O. microlepidota exhibits prominent C blocks at the telomeric regions of pairs 1 and 2 with a particularly large block on the long arm of pair one. Acrocentric pairs 6 and 7 possess very strong C positive blocks in the procentric region. Most of the microchromosomes are predominantly C-band positive (Fig. 3.16). The secondary constriction on pair one is also C-band positive.

3.5.4 The Pseudechis porphyriacus Karyomorph

I have examined chromosome data from all but one of the four Australian species of Pseudechis currently recognized by Cogger 1979. I was unable to obtain data from P. colletti. I do, however, present data here for specimens of an additional species, P. butleri, inhabiting Central Western Australia, that was recently described by L. Smith (1982) and is named in honour of the first collector, Harry Butler.
With the exception of *P. australis* all the species of *Pseudechis* studied have karyotypes closely resembling that of *P. porphyriacus* except, of course, for the species-specific variation in the morphology and banding character of the W chromosome (see also Fig. 4.1A and B) and some differences in the C-band character of the autosomes. *Pseudechis porphyriacus, P. butleri* and *P. guttata* all possess a diploid number of 36 with 16 macro and 20 microchromosomes. The three largest autosomal pairs again appear conserved as compared with most other Australian terrestrial elapids being respectively metacentric, submetacentric and metacentric in descending order of size. The Z chromosome, which is the 4th largest pair, is metacentric in *P. porphyriacus* and *P. butleri* while it appears slightly submetacentric in *P. guttata*. The W chromosome is larger than the Z being the third largest element in all species of *Pseudechis* studied and is metacentric in *P. porphyriacus*, subacrocentric in *P. butleri* and acrocentric in *P. guttata*. The remaining four pairs of macrochromosomes include one metacentric pair and three pairs of acrocentrics. Metacentric pair 5 and acrocentric pair 6 are very similar in overall length and the acrocentric pair even appears slightly longer than the metacentric in the figure offered here for *P. guttata*. The 20 microchromosomes are all acrocentric in all species, though many can be seen to possess minute short arms in the figure presented for *P. porphyriacus* (Fig. 3.17).

All species exhibit a secondary constriction on one arm of pair 1 and this constriction is C-band positive. Prominent centromeric heterochromatin is present on all macrochromosomes. Small telomeric blocks are evident on pairs 1, 2 and
Figure 3.17. *Pseudechis porphyriacus* (2n = 36, 16M/20m). Gross karyotypic (upper), C-band (middle) and G-band (lower) preparations. X's indicate overlaps and these areas should not be used in banding comparisons. Note prominent C positive centromeric regions and linear differentiation of the W chromosome into lighter and darker C positive regions (see also Fig. 4.1). This karyotype is proposed as the "primitive" condition in Australian elapids and is shared with a wide variety of species. See text sections 3.5.4 and 6.6.1.
Figure 3.18  Chromosomes of the genus *Pseudechis* A, C and E are gross karyotypic preparations, while B, D and F are C-banded preparations (A and B) *Pseudechis butleri* (2n = 36, 16M/20 m) note large C-band positive centromeric blocks (C and D) *P. guttata* (2n = 36, 16M/20m) note interstitial C-band on acrocentric pair 5. (E and F) *P. australis* (2n = 38 18M/20 m) here there appears to have been a fission of pair 3 giving rise to two acrocentric macroelements. See text sections 3.5.4 and 6.6.1. For G-band comparisons see figures 3.17 and 6.8.
the Z (Figs. 3.17, 3.18). *P. guttata* also possesses a procentric C-block on acrocentric pair 5 which explains the slightly larger relative size of this chromosome mentioned above (Fig. 3.18). *P. butleri* appears to display a relatively greater overall amount of heterochromatin, having particularly large heterochromatic blocks in the centromeric regions of the small metacentric pair and the three acrocentric pairs (Fig. 3.18). The majority of microchromosomes in this species appear almost completely heterochromatic. G-band data for the *Pseudechis* species are illustrated in this chapter and compared to other species in Chapter 6.

*Pseudechis australis* is distinguished from all other species in the genus, possessing a diploid number of 38 with 18 macrochromosomes and 20 microchromosomes (Fig. 3.18). This distinctive karyotype is the result of a Robertsonian rearrangement involving the metacentric pair 3 observed in the other species of *Pseudechis*. Thus *P. australis* possesses two small pairs of acrocentrics (pairs 8 and 9) in place of the metacentric pair 3 of the other species. The sex chromosome pair consequently appears to be the third rather than the fourth largest pair though the Z is equivalent in size to that of *P. porphyriacus* and *P. butleri* relative to the length of pair 1 in the respective species. G-band analysis unequivocally confirms the homology between pairs 8 and 9 of *P. australis* and pair 3 of the other *Pseudechis* species (Chapter 6).
3.5.5 Chromosomes of the Genus Cryptophis

Chromosome data is available from both species currently recognized in the genus Cryptophis. That from the four specimens of C. pallidiceps (see localities Table 3, Chapter 2) was obtained from slides prepared by J.J. Bull and kindly made available by him for this study. Unfortunately these offer only gross karyotypic information. On the other hand, I have studied C. nigrescens using C-, G- and N-banding as well as fluorescence, silver staining and in situ hybridization of an rRNA probe.

Cryptophis pallidiceps possesses a diploid number of 36. The macro and microchromosome categories are not clearly defined due to a gradual decrease in size from pair 5 through to pair 12 (Fig. 3.19A). Once again there is an apparent conservation of the three largest pairs of autosomes, at least in respect of gross morphological character, and a prominent secondary constriction is seen on one arm of pair 1. The Z chromosome is the fourth largest element and is submetacentric to subacrocentric, while the W is somewhat larger and subacrocentric with small but distinct short arms. Even in gross preparations, a series of constrictions are evident along the W chromosome. Pairs 5 through 10 are all acrocentric. Pair 11, which approaches microchromosome proportions, appears metacentric; the microchromosomes themselves are all biarmed in morphology.

Cryptophis nigrescens is perhaps the most variable snake species studied thus far in terms of its chromosome complement. It is the first snake species for which autosomal polymorphism has been demonstrated and also possesses at least
Figure 3.19. Gross karyotypic and C-banding data for a "typical" female Cryptophis nigrescens (2n = 40, 20M/20m in this specimen). Note large heterochromatic blocks on the acrocentric macrochromosomes. For G-band details refer to Figure 5.14. This species shows a high degree of chromosomal variation which is discussed and illustrated in greater detail in Chapter 5. See text sections 3.5.5, 5.2 and 6.6.1.
four W sex chromosome variants. A detailed description of the intraspecific variation is given in Chapter 5. Here I will describe only the basic karyomorph for the species which has been observed in at least some specimens from all populations and from which the numerous variants can be derived. The basic karyomorph consists of a diploid number of 40 with 20 macro and 20 microchromosomes. Unlike *C. pallidiceps* only the two largest pairs of autosomes are conserved. The metacentric pair 3 seen in *C. pallidiceps* and many other Australian elapids has clearly undergone a Robertsonian type of rearrangement resulting in its replacement by two acrocentric macrochromosomes (see G-band data Fig. 6.6 and Chapter 6). Such a rearrangement involving the metacentric pair 3 has already been described in the genus *Pseudechis* (Section 3.5.4). Because of the apparent fission of pair three the sex chromosome pair appears as the 3rd largest pair of chromosomes. This is followed by seven pairs of acrocentric macrochromosomes. Of the 10 pairs of microchromosomes the majority, but not all, appear biarmed.

In order to derive the *C. nigrescens* karyotype from that of *C. pallidiceps* it is necessary to assume a fission of one of the biarmed microchromosomes. By subtracting the 10 smallest pairs of chromosomes we see that the rearrangement of pair three accounts for all macroelements in *C. nigrescens*. The lack of demarcation between macro and micro-elements in *C. pallidiceps* may be due to heterochromatic addition to the microchromosomes, a phenomenon which is well documented in *C. nigrescens* (see Chapter 5).

Representative C- and G-band data is presented here for a
specimen of the 20M/20m karyomorph described for *C. nigrescens* (Fig. 3.19). Additional banding data is presented and discussed for the variants in this species in Chapters 5 and 6.

3.5.6 **Chromosomes of the Genus Acanthophis**

The species of the genus *Acanthophis* represent yet another group displaying a diploid number of 36 and composed of 16 macro and 20 microchromosomes with the three smallest macrochromosome pairs being acrocentric. I have examined all recognized species of *Acanthophis* though report here only on those forms inhabiting continental Australia and associated islands (namely, Recherche Archipelago specimen 206 and Groote Eylandt specimen 285). Differences in the position and composition of secondary constrictions and W sex chromosome morphology, as well as associated differences in C- and G-bandning and florescence, account for the distinguishing characteristics of the several species. I have also examined the chromosomes of specimens currently grouped with *A. antarcticus*, but which are distinguishable on gross external morphology and coloration, and these specimens too possess their own characteristic banding patterns in respect of the sex chromosome and the secondary constrictions. This genus is therefore discussed in some detail in Chapter 5 of this thesis. I present banding details for the genus in Figs. 5.3 and 5.4.
3.5.7 Chromosomes of the Genus Denisonia

Until 1980 this genus contained over 50% of the species of Australian elapid snakes. With the introduction of such genera as Austrelaps, Cryptophis, Unechis, Drysdalia and Suta, however, this genus has now dwindled to only four species. Even so, the controversy surrounding this assemblage continues, with some authors recognizing Austrelaps and Drysdalia but preferring "species-group" designations for all other forms (Storr, 1981; Coventry, 1971). Others opt for the recognition of the genus Denisonia in the strict sense which is held to contain only four species (Cogger, 1975, 1979; Smith, 1980).

In examining the chromosomes of three of the four species of Denisonia (sensu stricto) I find that two very distinct karyomorphs exist. Denisonia devisii and D. maculata both possess a diploid number of 34 with 14 macrochromosomes and 20 microchromosomes (Figs. 3.20). Pairs, 1, 3, 4 and 6 are metacentric or very slightly submetacentric. Pairs 2 and 5 are clearly submetacentric, while pair 7 is acrocentric. In D. maculata there are 3 pairs of metacentrics among the microchromosomes and most of the remaining elements appear biarmed (Fig. 3.20). The gross preparation offered for the female specimen of D. devisii does not clearly show the morphology of the microchromosomes but gross and C-band preparations of several male specimens suggests that the microchromosomes are essentially similar to those of D. maculata.

C-banding demonstrates prominent centromeric blocks on all macrochromosomes and most microchromosomes. At least two
Figure 3.20 (above)  Gross karyotype of a male *Denisonia maculata* (2n = 34, 14M/20m), (below in descending order). Gross karyotypic, C-band and G-band preparation of *D. devisii* (2n = 34, 14M/20m). Note the sex chromosome pair is the fifth largest pair. These two species of *Denisonia* are more similar to each other chromosomally than they are to *D. punctata* (2n = 30). The latter appear most similar to *Suta* (see Fig 3.22). Refer to text sections 3.5 and 6.6.1.
pairs of microchromosomes appear completely C-band positive. Clear telomeric C-bands are located on both arms of all macroelements being most intense on pairs 2, 3, 4, 5 and 7. There is an additional procentric C-block on pair 4.

It is very interesting to note that, unlike most other Australian elapid species, the heteromorphic sex pair in *D. devisii* is clearly the fifth largest pair, the W is acrocentric and much larger than the typically submetacentric Z. I had access to gross karyotypic data from only a single male specimen of *D. maculata* and so the sex pair cannot be confidently demonstrated. It is worth reiterating, however, that all the macroelements agree completely with those described above for *D. devisii*.

G-band data is presented here for *D. devisii* (Fig. 3.20) utilized in species comparisons in Chapter 6 of this thesis.

*Denisonia punctata* contrasts sharply to all the other species of *Denisonia* described above, in possessing a diploid number of 30 consisting of 16 macrochromosomes and 14 microchromosomes (Fig. 3.21). The largest microchromosome pair is half the size of the smallest pair of macrochromosomes yet is still considerably larger than the microchromosomes of many other elapid species.

The first three pairs of autosomes again appear conserved, pairs 1 and 3 being metacentric and pair 2 submetacentric. There is an obvious secondary constriction on pair one. The fourth largest pair of chromosomes constitutes the sex pair with a slightly submetacentric Z and a much larger submetacentric W. Pair 5 is submetacentric and pairs 6, 7, 8 are
subtelocentric with short arms. Pairs 9, 10, 11 and 13, which fall into the microchromosome category, are all metacentric or only slightly submetacentric. The remaining microchromosomes are also biarmed but are more submetacentric to subtelocentric. This karyotype is almost identical to that seen in the genus Suta (Fig. 3.21) and some species of the genus Unechis, at least in respect of the gross morphology of its autosomes. Unfortunately G-band data is not currently available for D. punctata to compare with that for Suta and Unechis.

3.5.8 **Chromosomes of the Genus Suta**

I have examined a number of specimens of the monotypic genus Suta utilizing a variety of banding and fluorescence techniques. As mentioned above, the gross karyotypic appearance of the autosomes is remarkably similar to that described for D. punctata (Fig. 3.21). *Suta suta* does differ from *D. punctata* in having a more submetacentric *Z* chromosome. This may, however, be subject to intraspecific variation in *Suta* (see below).

Intraspecific variation is certainly evident between female specimens numbers 30 and 296 in the morphology of both the *W* chromosome and the smallest micro-element. In specimen 30 the *W* chromosome is shorter relative to chromosome 1 and the *Z* than is the *W* of specimen 296. This is due to a much smaller short arm in the subtelocentric *W* of 30, as compared to the large submetacentric *W* of specimen 296. The long arm of the *W* appears unchanged (Fig. 3.21). Additionally the
Figure 3.21. Gross karyotypes of *Suta suta* specimens 30 (upper) and 296 (mid) and *Denisonia punctata* (lower). All possess a diploid number of 30 with 16 macro- and 14 microchromosomes. Note that in *Suta* while the W chromosomes vary in morphology both possess the subacrocentric morph of the heteromorphic Z found in males of the species. (See Fig. 3.22). It is interesting to note that the karyotype of *D. punctata* is more similar to *Suta* than to *D. maculata* and *D. devisii* which possess a 2n of 34 (14M/20m) seen in Figure 3.20. Refer to text sections 3.5.8, 3.5.7, 6.2.1(2) and 6.6.1 and Fig. 4.1B.
smallest micro-element in specimen 30 appears biarmed, whereas in specimen 296 it is acrocentric.

Prominent C-bands appear restricted to the sex chromosome pair in *Suta* with even the centromeric C-bands appearing quite faint. The secondary constriction on pair one is C-band negative. The W chromosome shows distinct linear differentiation in both C- and N-bands. DAPI fluorescence demonstrates a very intense DAPI positive series of blocks over the distal half of the long arm of the W (Fig. 4.4). Incidentally the secondary constriction on pair 1 is DAPI negative.

Another notable feature demonstrated by C-banding is the heteromorphic character of the Z chromosomes of the males. One Z consistently appears slightly shorter and more submetacentric than its homologue. Both Z chromosomes possess an intense C-block at the telomeric end of the short arm but in the larger Z, with a more median centromere, the C-block is almost twice the size of that of its homologue (Fig. 3.22). Even more intriguing is the fact that females all appear to possess only the smaller more submetacentric Z. Whether this will prove to be a consistent feature of this species can only be determined with larger sample sizes but the data is certainly suggestive. G-bands incidently show this C-block to be G negative and clearly heteromorphic.

3.5.9 **Chromosomes of the Genus *Unechis***

The generic name *Unechis* was expanded by Cogger (1975, 1979) to accommodate those species of the once large genus *Denisonia* which, as described in Chapter 1, Worrell had
Figure 3.22. C-banded preparations of the macrochromosomes of two male specimens of *Suta suta*. Note the heteromorphism in gross morphology and heterochromatic content between the two Z chromosomes in each male (underlined). Refer to text sections 3.5.8 and 6.2.1(2).
allocated to Parasuta, Unechis and Cryptophis. McDowell (1970) placed all the species we now know as Unechis in the genus Suta along with D. fasciata and D. punctata. Chromosomal data presented here demonstrate the genus Unechis is a composite of three distinct karyomorph groups. Consequently I believe that the taxonomic arrangements listed above still obscure the true relationships of the species involved.

The first and largest of the karyomorph groups found in Unechis is very similar to that described above for Suta and D. punctata, at least in gross karyotypic details. This karyomorph is found in all the "black headed" Unechis of the U. gouldii species group (see Storr, 1981; Coventry, 1971). More specifically I have examined all but one species of Unechis (namely U. spectabilis) and found a 2n = 30 karyomorph in U. dwyeri, U. flagellum, U. gouldii, U. nigriceps (= brevicaudus) and U. monachus (Figs. 3.23, 3.24, 3.25, 3.26). As in Suta and D. punctata the 30 chromosomes can be divided into 16 macro and 14 micros, though again the micro-elements are relatively large in comparison to other elapid species. The gross morphology among this group of Unechis species is identical, save for the W sex chromosome. Slight differences appear in C-band character. U. dwyeri exhibits prominent telomeric C-blocks on all macrochromosomes, including the Z, and centric C-bands on all but pairs 2 and 3 (Fig. 3.23). U. nigriceps shows only the centric blocks and a single telomeric block on one arm of the Z (Fig. 3.24). It is interesting to note that the telomeric heterochromatin on the Z chromosome in these Unechis species results in an obvious G-band negative region similar to that seen in Suta. G-band data is
Figure 3.23. *Unechis dwyeri* (2n = 30, 16M/14m). Gross karyotype (upper), C- (mid) and G-band (lower) preparations. Sex chromosomes are underlined. Refer to text sections 3.5.11 and 6.6.1.
Figure 3.24 (above)  Gross karyotypic and C-band preparations of *Unechis nigriceps* (= *brevicaudus*) (2n = 30 16M/14m) (below).  Gross karyotype of *U. flagellum* (2n = 30 16M/14m).  Refer to text sections 3.5.9 and 6.6.1.
Figure 3.25  *Unechis carpentariae* (2n = 36). Gross karyotypic (above), C-band (middle) and G-band below preparations. Note this species is completely unlike the species of the *Unechis gouldii* group in diploid number, morphology and banding details (compare with Figures 3.23 and 3.24). More detailed G-band comparisons are available in 6.7. See text sections 3.5 and 6.11.
illustrated here for *U. dwyeri* and a comparison of G-bands between the 2n = 30 *Unechis* and *Suta* is illustrated in Chapter 6 (Fig. 6.5).

Unlike the large W chromosome of *Suta* and *D. punctata* that of the *Unechis* species is similar in length to the Z and is almost homomorphic. C-banding demonstrates, however, a darker C-positive block in the centric region of the heterochromatic W of *U. dwyeri*. This area is clearly G-band positive (see Fig. 6.5) and DAPI positive in fluorescence (Fig. 4.4). Female specimens were not available for banding in all the "black headed" *Unechis* species but those that were analysed showed species-specific banding patterns of the W sex chromosome.

The remaining two species in the genus, *U. carpentariae* and *U. nigrostriatus*, possess distinctive karyomorphs quite unlike the "*U. gouldii* species complex" just discussed and differing somewhat from one another. In *U. carpentariae* the diploid number is 36. There is a gradual decrease in size from pair 5 to pair 12, making the distinction between macro and microchromosome categories ill defined. The first three pairs of macrochromosomes appear conserved as compared to the "*U. gouldii* species complex" and indeed most other elapid species. The sex chromosome pair is again the fourth largest, with a submetacentric Z and a larger acrocentric W. Pair 5 is acrocentric with a minute short arm. The remaining autosomes, including the micro-elements, are acrocentric with the exception of one metacentric pair which is 10th or 11th in descending size order. It should be noted here that the gross karyotype of *U. carpentariae* is essentially identical to that of *Cryptophis pallidiceps* even with regard to the morphology of
the Z chromosome (compare Figs. 3.25 and 3.19). Unfortunately, as mentioned earlier, banding details are not available for C. pallidiceps. G-bands are, however, available for U. carpentariae and in Chapter 6 these are compared with the "U. gouldii" species group on the one hand, and C. nigrescens, the only other member of the genus Cryptophis, on the other hand (Fig. 6.6).

The C-banding in U. carpentariae also differs from the other Unechis species. Faint centric C-blocks occur on pairs 1, 2, 3, 7, 8 and the Z chromosome, while more prominent centric blocks are seen on acrocentric pairs 9 and 12 and metacentric pair 11. Faint procentric blocks are obvious on both arms of pair 5, the acrocentric pair possessing short arms. A very large and dense C-positive block is seen in the procentric region of acrocentric pair 6. The W-chromosome appears darkly heterochromatic but prometaphase cells show some segmentation into lighter and darker regions.

The remaining species of Unechis, U. nigrostriatus, possesses 34 chromosomes and, as in U. carpentariae, a gradual decrease in size from pair 5 to 12 makes the distinction between macro and microchromosomes unclear (Fig. 3.26). While many of the chromosome pairs appear unchanged between these species, pair 6, 7, 9, 10 and 12 are all biarmed in U. nigrostriatus and acrocentric in U. carpentariae. Additionally Unechis nigrostriatus possesses one metacentric pair of microchromosomes in place of two of the acrocentric micro pairs seen in U. carpentariae, thus explaining the difference in diploid number. Pairs 6 and 7 in U. nigrostriatus differ from those of U. carpentariae in the
Figure 3.26. *Unechis nigrostriatus* (34, 24M/10m). The gross karyotype of this species is quite unlike that of other members of the genus and with *U. carpentariae* differs radically from the 2n = 30 "*U. gouldii* species complex". See text sections 3.5.11 and 6.6.1.
presence of short arms, while pairs 9, 10 and 11 are meta-centric in the former and acrocentric in the latter (compare Figs. 3.25 and 3.26). The absence of banding data for the single male specimen of U. nigrostriatus prohibits determining whether these differences are due to heterochromatic addition or pericentric inversion. In the light of these differences, and in the absence of banding data from both Glyphodon and critical genera inhabiting Papua New Guinea, one cannot determine with certainty whether U. nigrostriatus lies closer to U. carpentariae or to some as yet unstudied species. It is clear, however, that both species are not closely related chromosomally to the Unechis gouldii species complex.

3.5.10 The Chromosomes of the Genus Purina

The genus Purina (sensu Cogger, 1979) has had a turbulent taxonomic history. Worrell divided Furina (sensu Boulenger) into two genera, Brachyaspis for diadema and Lunelaps for christianus. McDowell subsequently showed the two species to be congeneric and held that the use of the generic name Brachyaspis was invalid. Cogger (1975) included F. christianus in F. diadema. Storr (1981) has more recently upheld the morphological validity of the christianus form and resurrected the name F. ornata to describe it. Without further assessment of the characters, but presumably because of McDowell's (1967) suggestion that Purina is related to Glyphodon rather than Aspidomorphus (including Cacophis), Storr has also included within Purina two species of the genus Glyphodon (barnardi and tristis) and the troublesome species
warro, previously placed in Simoselaps. Storr's reasons for omitting the third species of Glyphodon (dunnalli) were not stated.

I have analysed the chromosome complements of Furina diadema and F. ornata and find them cytologically distinguishable. Some individuals of Furina diadema possess a diploid number of 34 with 14 macro and 20 microchromosomes. Pairs 1 to 3 appear conserved. Pair 4 is the sex pair with a very unusual acrocentric Z and a larger subacrocentric W. Pairs 5 and 6 are metacentric and pair 7 is acrocentric (Fig. 3.27). The morphology of the microchromosomes is not clear in the illustration (Fig. 3.27) presented for this morph but there appear to be both biarmed and acrocentric elements present. C-banding is available for this form and, in addition to centric C-blocks on the autosomes, the W exhibits lighter and darker C-positive regions (see Fig. 3.27).

Specimens from the northern extreme of the range, identified as F. ornata, also possess 34 chromosomes with 14 macro and 20 micro-elements but are easily distinguishable by the presence of an acrocentric pair 2 (Fig. 3.27). This appears to be equal in size to the pair 2 seen in Furina diadema and other Australian terrestrial elapids and is probably the result of a pericentric inversion. The sex pair also differs from that seen in Furina diadema. The Z exhibits a more characteristic median centromere position, while the W is subacrocentric and as large as the second largest autosome. Five of the 10 pairs of microchromosomes are clearly metacentric to submetacentric. All other autosomes appear identical to those of Furina diadema (Fig. 3.27).
Figure 3.27. Gross karyotypic data on Furina. *Furina ornata* (christiana) (2n = 34, 14M/20) differs from the *Notechis* karyotype by an apparent inversion in pair 2. It also possesses an unusually large subacrocentric W chromosome (top). *Furina diadema* is more like *Notechis* in gross karyotype in that it possesses the typical submetacentric pair 2 (mid). The Z chromosome however is acrocentric, a morphology unique among Australian elapids. Three specimens of *Furina* from north central N.S.W. are unlike both the above in possessing a diploid number of 36 with 16 macro and 20 microchromosomes (bottom). Unfortunately banding data is not available for these specimens. Refer to text sections 3.5.10 and 6.6.1.
There exists yet another karyomorph within the genus Furina which I have identified in three specimens of F. diadema from different localities in North central New South Wales (specimens: 119, Macquarie Marshes, N.S.W.; 316, near Dubbo, N.S.W.; 200, Burke, N.S.W.). All of these possess a diploid number of 36 with 16 macro and 20 microchromosomes (Fig. 3.27). The centromere of the Z chromosome is median in position, more like that of F. ornata than other F. diadema. There also exist an additional pair of acrocentric macro-chromosomes with minute short arms. Without further banding data on these forms it is not possible to determine whether such a karyotype has been derived from other Furina karyotypes by Robertsonian rearrangement of one of the larger micro-elements followed by heterochromatic addition, though this seems a likely hypothesis.

In conclusion, it is apparent that Furina is composed of at least three karyotypic forms and the two currently recognized species, (F. diadema and F. ornata), are clearly distinct chromosomally. As no specimens of Glyphodon have been examined I cannot test the validity of Storr's (1981) decision to include two of the species of that genus in Furina. However, as will be apparent from the data presented below, the species S. warro is certainly not closely related chromosomally to any of the Furina karyomorphs described here.

3.5.11 Chromosomes of the Genus Hemiaspis

The genus Hemiaspis as we now know it, consists of two species, H. signata and H. damelii (Cogger, 1975, 1979). H.
damelii was first removed from the large genus Denisonia (sensu Boulenger) by Worrell (1961) and placed in a new genus, Drepanodontis. H. signata was included, along with the Copperhead, in the genus Austrelaps by the same author (Worrell, 1963). McDowell (1967) demonstrated an acute similarity between the two species in venom gland musculature, dentition and skull morphology, placing them together in the genus Drepanodontis. Substantial taxonomic and phylogenetic significance was attributed to this genus when McDowell (1967, 1972, 1974) suggested that the hydrophiid sea snakes could be derived from Australian terrestrial elapids via his "Demansia group" (see Chapter 1, section 1.1.2) and specifically Drepanodontis (= Hemiaspis) and Rhinoplocephalus. As we shall see in subsequent discussion, both the chromosomal data presented here and the MC'F data of Cadle and Gorman (1982), demonstrate that the "Demansia group" of McDowell is in some sense a composite of unrelated genera; Demansia itself being an "out" group as compared both with other genera of the grouping and Australian elapids in general.

I have examined banding data from both species in the genus Hemiaspis in some detail and found them to differ by several Robertsonian rearrangements. H. damelii appears to possess the more conserved of the two karyotypes in exhibiting a diploid number of 34 with 16 macro and 18 microchromosomes. The first three pairs are obviously conserved, having median, submedian, and median centromere positions respectively. The sex pair is the fourth largest pair with a slightly submetacentric Z and a smaller acrocentric W. Pair 5 is metacentric, while pairs 6 and 7 have subtelocentric
centromeres and pair 8 has a terminal centromere. There are several pairs of biarmed elements among the microchromosomes, the largest being metacentric (Fig. 3.28). In addition to faint centromeric bands on all chromosomes, including the micro-elements, all the autosomal macrochromosomes possess obvious telomeric C-bands on both arms (Fig. 3.28). The acrocentric W chromosome is composed of largely dark staining heterochromatin though a segmentation of this condition is evident.

The karyotype and banding pattern of *H. signata* has undergone considerable change compared both with its congenitor and other terrestrial elapids. It possesses a diploid number of 40 with 20 macro and 20 micro-elements. There are only three pairs of obviously biarmed elements in the genome. These are the sub-metacentric pair 1, the slightly sub-acrocentric Z, which is the fourth largest element, and a subacrocentric pair which is in the same size class as pairs 5 through 8. All the remaining elements, including the 20 microchromosomes, are acrocentric. The high number of acrocentric elements in the karyotype is the result of at least two fission events. Thus metacentric pair 1, which possesses the NOR secondary constriction observed in all other Australian elapids, has undergone a centric fission giving rise to acrocentric pairs 2 and 3 in *H. signata* and resulting in the subacrocentric pair 2 of other elapids now becoming the largest element in the *H. signata* karyotype. G-banding and silver staining demonstrate that, apart from fission, these elements are not otherwise changed, and the prominent secondary constriction is apparent on acrocentric pair 2 (Fig. 3.29).
Figure 3.28  *Hemiaspis damelii* (2n = 34 16M, 18m). Gross karyotypic (above) and C-band (two below) preparations of the macrochromosomes. Note the C-bands of this species do not differ markedly from those of *H. signata* though two fission/fusion events are necessary to explain the difference in diploid number between these species. (Compare with Fig. 3.29). See text sections 3.5.11 and 6.6.1.
Figure 3.29. *Hemiaspis signata* (2n = 40, 20M/20m or 22M/18m). Though this species is described in the text as possessing 20 macro and 20 microchromosomes the two gross karyotypic figures offered here (A & B) demonstrate that the macro and micro element categories are ill defined and could just as easily be described as 22M/18m. (C) Shows that this species does not possess the large C-blocks seen on the acrocentrics of *Cryptophis nigrescens* (Chapter 5) and the secondary constriction of pair 2 is C-band negative. This constriction is also G-band negative (D). See also sections 3.5.11 and 6.6.1 in the text.
Likewise there has been a centric fission, involving the normally metacentric pair 3 seen in other Australian elapids, which has given rise to two small acrocentric pairs. This is confirmed through G-band analysis. An additional Robertsonian rearrangement involving two acrocentric micro-elements of *H. signata* on the one hand, and the metacentric micro-element on the other, would explain the difference in the number of microchromosomes between the two species. The direction of this change cannot, however, be determined with the same confidence as the other rearrangements present in *H. signata* because of the apparent plasticity exhibited by the microchromosomes of some genera of Australian elapids. C-bands in *H. signata* do not differ markedly from those of *H. damelii*, discussed above, except that not all acrocentric macroelements possess prominent telomeric heterochromatin and there is an unusually large procentric C-block on the subacrocentric element. The secondary constriction on pair 2 is obviously both C-band negative (Fig. 3.29) and DAPI negative (Fig. 4.3). The W chromosome of *H. signata* shows clear linear differentiation in DAPI fluorescence (Fig. 4.3) though this is not apparent in all C-band preparations.

3.5.12 Chromosomes of the Genus Simoselaps

As outlined in the Introduction (Section 1.1.2) Storr's (1967) revision of the genus *Vermicella* led to the inclusion within it of species from the Australian genera *Narophis*, *Melwardia*, *Brachyurophis*, *Rhynchoelaps* and *Rhinelaps*. McDowell (1969) subsequently agreed with Storr's grouping of
these species with the exception of annulata, the type species of Vermicella. Consequently he placed all the other species under the generic name Rhynchoelaps. Cogger (1975) has considered Rhynchoelaps a "nomen nudum" and suggested the name Simoselaps for all these species with the exception of bimaculata and calonota which he considers to belong to Neelaps.

I have obtained chromosome data from only two species of the genus Simoselaps, namely S. bertholdi and the controversial S. warro, together with Neelaps bimaculata (see below). The gross karyotype, as well as C- and G-banding data (Fig. 3.30) is presented here from a number of specimens of S. bertholdi (see species examined, Chapter 2, Table 2.1). This species possesses a diploid number of 38 with 18 macro and 20 microchromosomes. Again pairs 1 to 3 are conserved, being metacentric, submetacentric and metacentric respectively with an obvious secondary constriction on pair 1. The sex chromosomes are the fourth largest pair, the Z being submetacentric, while the W is acrocentric, and of approximately equal length. Pairs 5 to 9 are acrocentric with pairs 6, 8 and 9 possessing minute short arms. The twenty microchromosomes are acrocentric to subacrocentric. This gross karyotypic morphology could conceivably have been derived from a 2n = 36 karyomorph in which the three smallest pairs of autosomes were acrocentric, and which is seen in a number of genera (see Section 3.5.15 below), by a centric fission of the typically metacentric pair 5 into two small pairs of acrocentrics. This hypothesis is explored further utilizing G-band comparisons in Chapter 6. Faint C-bands appear at both the centromeres and
Figure 3.30. (A) Simoselaps warro (2n = 38,18M/20m) gross karyotypic preparation. S. bertholdi (2n = 38,18M/20m) (B) gross karyotypic preparation (C) C-banded preparation (D) G-banded preparation. See text sections 3.5.12 and 6.6.1
telomeres of all macrochromosomes, including the Z. The W chromosome is predominantly C-band positive.

Perhaps one of the most taxonomically controversial species of Australian terrestrial elapids is S. warro. Storr in 1979 considered it only doubtfully included in Vermicella (= Simoselaps, Neelaps and Vermicella of Cogger 1975) and referred to warro as Cacophis following the original designation of DeVis. More recently Storr (1981) has included it in the genus Furina. Chromosomally it appears identical to S. bertholdi in gross karyotype (Fig. 3.30). As such the karyotype is totally unlike that of Cacophis or any of the Furina karyomorphs described above. This suggests to me that chromosomally the affinities of S. warro lie clearly with Simoselaps and not to any of the other genera with which it may have been aligned in the past. From the most recent morphological and ecological study of the species groups within Simoselaps and Neelaps (Shine 1982, in press) it is clear that S. warro resembles the "S. bertholdi group" in being saurophagus and lacking the exaggerated shovel-like snout and dentitional adaptations of the other species.

3.5.13 Chromosomes Of Echiopsis, Neelaps, Rhinoplocephalus and Vermicella

In this section I consider the gross karyotypic data from four of the genera which appear to share the same karyomorph but for which, regrettably, I do not have adequate banding data. Two of these are monotypic and the other two have only 2 species each. If one excluded the species-specific W sex
Figure 3.31. Gross karyotypes of *Rhinoplocephalus bicolor*, *Vermicella annulata* and *Echiopsis curta* given in descending order. All possess a diploid number of 36 with 16 macro and 20 microchromosomes and with the three smallest pairs of macrochromosomes being acrocentric. They vary in the morphology of the W chromosomes and some of the micro-elements. See text sections 3.5.13 and 6.6.1.
chromosome morphology, and perhaps also the centromere position in some of the microchromosomes, then these species appear identical in gross karyotype to the many species of *Pseudechis* and *Acanthophis* for which a variety of banding data is reported here. The gross karyotype for all of these again consists of a diploid number of 36 with 16 macro and 20 microchromosomes (Figs. 3.31 and 3.32). Pairs 1 through 3 are metacentric, submetacentric and metacentric respectively. The sex chromosome pair is the fourth largest with a submetacentric Z in all six species of the four genera described here. The W chromosome in *Vermicella annulata* is subacrocentric.

Karyotypic data was available for males only of *Rhinoplocephalus*, *Echiopsis* and *Neelaps bimaculata*. Pair 5 is metacentric and pairs 6 through 8 are acrocentric. As mentioned above, the 20 microchromosomes vary in the number of biarmed elements present.

This group of genera represents perhaps the most diverse species that have been shown to share the same karyomorph. The fossorial *Neelaps* is generally considered closest to *Simoselaps* and Storr (1967) considers *Vermicella annulata* to be related to both these genera. *Echiopsis* is often considered to be closest to *Acanthophis*, a relationship which is supported by the similarity of their gross karyotypes. The affinities of *Rhinoplocephalus* are unclear, though some authors have suggested that they are "primitive" among Australian elapids. It will be noted in the following section that one of the species of *Cacophis* for which banding data is available (*C. kreffti*) also shares this gross karyotype, while all the other species of *Cacophis* examined possess short arm differences in
Figure 3.32. Gross karyotypes of a male *Neelaps bimaculata* (2n = 36:16M, 20m). See text sections 3.5.13 and 6.6.1.
pairs 6 to 9. The four genera covered in this section have in the past been considered in two taxonomic groups, (1) *Echiopsis* and *Acanthophis* and (2) *Neelaps*, *Vermicella* and possibly also *Rhinoplocephalus*. However, since *Acanthophis* and *Pseudechis* also share this same gross karyotype with $2n = 36$, and *Cacophis* can be easily derived from it, it would seem more logical to suggest that this karyomorph in fact includes a wide variety of quite unrelated species. As such it would fit the criteria outlined in Section 1.2.1, Chapter 1, for defining an ancestral karyomorph. Thus, if a number of apparently unrelated species share the same karyomorph it is more reasonable to assume they have conserved a primitive condition than to suggest that they converged upon this karyomorph from a number of ancestral conditions and resulted in the same banding patterns. Clearly, the maintenance of a primitive character state need not denote relationship among those sharing it. The candidacy of this and other karyomorphs for the archetypic condition will be assessed in the concluding section of this thesis dealing with the phylogenetic implications of the data.

3.5.14 The Chromosomes of the Genus *Cacophis*

McDowell (1967) is primarily responsible for delineating the genus *Cacophis* as we know it today. The three species *kreffti*, *harrietae* and *squamulosus* have been historically divided into a number of genera, including *Furina*, *Glyphodon* and *Aspidomorphus*, but the suite of characters analysed by McDowell (1967) demonstrate them to be congeneric. Of these three species I have examined two, *C. kreffti* and *C.
squamulosus, using a variety of banding techniques, as well as in situ hybridization and fluorescence. As described in the preceding section, one of these species, C. kreffti, shares the same gross karyotypic appearance of the $2n = 36$ (16M/20m) karyomorph with three pairs of small acrocentric macrochromosomes, also seen in a wide variety of presumably unrelated species of Australian terrestrial elapids (compare Fig. 3.33 with Fig. 3.31). The other species, C. squamulosus, differs only in the presence of short arms on the three smallest macrochromosomes (Fig. 3.34). Thus pair 6 is submetacentric, while pairs 7 and 8 are subacrocentric. C-bands suggest these short arms cannot all be explained by heterochromatic addition (Fig. 3.34) and inversions are thus a more likely explanation. A detailed G-band comparison between the Cacophis species (Fig. 3.33 and Fig. 3.34) and the other elapids, with a diploid number of 36 is presented in Chapter 6.

3.5.15 Chromosomes of the Genus Hoplocephalus

This genus contains the three species of broadheaded snakes all of which, unlike other Australian elapids, possess keeled ventral scales and are adapted for climbing. This highly specialized group is one of only two genera of Australian elapids that has not been altered taxonomically in the past two and a half decades, the other being the monotypic Rhinoplocephalus. Chromosomally they also seem to form a unique and cohesive group. I have analysed banding data from both sexes of all three species recognised within the genus. All three possess a diploid number of 28 in the males. Two of
Figure 3.33. *Cacophis kreffttii* (2n = 36 16M, 20m). This species closely resembles the 2n = 36 karyomorph proposed as ancestral for the Australian elapid radiation in that the three smallest macroelements are acrocentric. Note centric and telomeric C-bands (below) on all macrochromosomes. There is also an indication of interstitial C-bands on the acrocentrics. The G-bands of *Cacophis* are compared to other 2n = 36 Australian elapids in Chapter six. Refer also to texts, sections 3.5.14 and 6.6.1.
Figure 3.34. *Cacophis squamulosus* (2n = 36, 16M/20m). This species differs from its congenitor in possessing short arms on the three smallest macroelements (compare with Figure 3.33) and the apparent absence of telomeric heterochromatin. Thus, aside from the W-sex chromosome, there appears to be only small amounts of centromeric heterochromatin. An additional example of the C-banded W chromosome is provided here to illustrate the submetacentric character not clearly visible in the cell provided. See also text sections 3.5.14 and 6.6.1. The G-band data for this species is provided in Chapter 6.
them, *H. bitorquata* and *H. bungaroides*, exhibit a diploid number of 27 in the females. I have interpreted this to be due to the translocation of an autosome from the micro-element category onto the W sex chromosome. This is discussed in some detail in Chapter 4, Section 4.4.

In all three species the 28 chromosomes can be divided into 14 macro and 14 micro-elements. All appear similar in respect of the autosomal macrochromosomes though there is interspecific variation in both microchromosome size and centromere position. In *H. stevensi*, as in the other species, pairs 1 through 3 are again conserved, being metacentric, submetacentric and metacentric. Pairs 4 and 5 are almost identical in overall length, but I designate pair 4 to be the sex pair. The Z is slightly submetacentric, while in W in *H. stevensi* is subacrocentric and larger than the Z. Pair 5 is also slightly submetacentric, pair 6 is subacrocentric with short arms. Pair 7 is metacentric. Of the seven pairs of micros the five largest pairs are clearly metacentric. Of the smallest two pairs, one appears biarmed while the other does not (Fig. 3.35B). Distinct C-blocks are apparent at the centromeres of all the autosomes including the micro-elements and the Z. The secondary constriction on pair 1 is C-band positive (Fig. 3.35C). *H. bungaroides* is similar to the karyomorph described for *H. stevensi* except for the translocation of one of the smallest acrocentric micro-chromosomes to the W (Fig. 3.35A and Section 4.4 Chapter 4). The W chromosome itself is acrocentric and about equal in size to the Z. There appear to be only three pairs of clearly metacentric microchromosomes. *H. bitorquatus* is most similar
Figure 3.35. (A) - Gross karyotype of *Hoplocephalus bunguroides* female (2n = 27; 14M/13m) indicating an autosomal (microchromosome) translocation to the W sex chromosome, as seen in *H. bitorquatus* (Fig. 3.36). (B) - Gross karyotype of *H. stephensi* (2n = 28; 14M/14m). (C) - C-band data on a female specimen of *H. stephensi* (2n = 28; 14M/14m) with a simple Z W sex determining system. Note small centromeric C-bands on all chromosomes and the C positive secondary constriction on pair 1, as well as the linear differentiation of the C-band pattern of the W sex chromosome. The genus *Hoplocephalus* forms a cohesive and distinct karyomorph group with a low diploid number. See text sections 3.15 and 6.6.1.
to *H. bungaroides*, with which it shares the microchromosome translocation to the W. In *H. bitorquatus*, however, the acrocentric W possess a minute short arm and is considerably larger than the Z. This species also differs in having an acrocentric pair 8 which is the largest micro-element and which is metacentric in the other two species (Fig. 3.36). There are only two pairs of metacentric microchromosomes in *H. bitorquata*. Representative C and G-band data is presented here for the *Hoplocephalus* and detailed comparisons are to be found in Chapter 6.

3.5.16 The Demansia Karyomorph

Species of the Whip Snake genus *Demansia* were originally considered congeneric with the Brown Snakes. This was altered by Worrell's separation of the Brown Snakes into the genus *Pseudonaja*. Cogger (1975) recognized four species of Whip Snakes. Storr (1978) brought the total to seven taxa by describing an additional species and several subspecies. I have analysed the chromosomes of five of the species of *Demansia* (*D. olivacea*, *D. papuensis*, *D. atra*, *D. psammophis* and *D. torquata*) and found them to represent a cohesive group with a unique karyomorph. All possess a diploid number of 42 with 22 macro and 20 microchromosomes. There is little variation between species except in the size of the W chromosome. All macroelements are biarmed and there is no apparent conservation of the larger macrochromosomes as compared to many other Australian elapids. The sex chromosomes, pair 4, include a slightly sub metacentric Z and a subacrocentric W, which may be
Figure 3.36. *Hoplocephalus bitorquatus* (2n = 28, 14M/14m 27 14M 13m) illustrating gross karyotypic data on both male and female specimens (upper) and two examples of the G-band data on female specimens (lower). These karyotypes suggest an autosomal (microchromosomal) translocation to the W sex chromosome resulting in the reduced diploid number in females. This reduced diploid number is the lowest yet encountered among Australian elapids. See text sections 3.5.15 and 6.6.1.
either smaller or larger than the Z depending on the species (Fig. 3.37). Pair 1 in all species is submetacentric, pairs 2, 3 and 5 are subacrocentric, while pairs 6 through 11 are metacentric or very nearly so. The 20 microchromosomes are predominantly acrocentric though many show distinct short arms. There are 3 pairs of micro-elements that could be described as submetacentric. C-banding is restricted to centromeric regions. Thus the short arms of the larger macrochromosomes are clearly not derived by heterochromatic addition. G-bands are presented here for *D. psammophis*.

What is apparent from the analysis of the chromosomes of *Demansia* is that all the species of this genus possess a karyomorph quite unlike that of any other Australian elapid, so that they cannot easily be derived from any other known karyomorph. It is worth noting here that the MC'F work of Cadle and Gorman demonstrated *Demansia* was distinctive among Australian elapids in displaying the greatest immunological distance from all the elapid reference species they used, so that on this basis too they appear to be phylogenetically distinct.

3.5.17 **Chromosomes of the Genus Pseudonaja**

The genus *Pseudonaja* was resurrected in the early 1960's by Worrell to house the "Brown Snakes" which, prior to his revision, were included with the Whip Snakes in the genus *Demansia*. Though the genus itself appears well defined, the species within it certainly are not. In his classical work Cogger (1979) points out that the present classification is
Figure 3.37. Gross karyotypic preparations of *Demansia atra*, *D. psammophis* and *D. olivacea* in descending order. All possess a diploid number of 42 with 22 macro and 20 microchromosomes. The genus *Demansia* is unlike any other Australian elapid genus in its chromosome composition. See text sections 3.5.16 and 6.6.1.
"uncertain and unreliable". There is a "high level of individual and population variability in colour pattern and scalation. Most species are probably composite". Indeed, in his review of the *Pseudonaja* from the Northern Territory, Gillam (1979) recognized twelve variants of the species *P. nuchalis* on the basis of colour and back pattern. In the light of this situation, it is interesting to note that the genus *Pseudonaja* displays the most variable chromosome number of any Australian elapid genus. More specifically *P. nuchalis* is clearly a composite of at least three distinct karyomorph groups. I have examined chromosome banding data from all currently recognized species of the genus *Pseudonaja* and data is presented here under individual species headings:

(1) *P. textilis* (2n = 38; 18M, 20m)

All specimens of *P. textilis* possess a diploid number of 38 with 18 macro and 20 microchromosomes (Fig. 3.38). Metacentric pair 1 possesses a prominent secondary constriction as seen not only in all *Pseudonaja* but indeed in all but one of the genera of Australian terrestrial elapids, namely *Demansia*. Pair 2 is submetacentric and pair 3 is metacentric. The sex chromosomes are the 4th largest elements with a submetacentric Z and W of approximately equal total length, though with a slightly different centromere position. Pairs 5 through 9 form a separate size class with a gradual decrease in size making individual size designations difficult. Pairs 5, 7 and 9 are subacrocentric, all possessing minute short arms though with pair 5 exhibiting the largest of these. Pairs 6 and 8 are submetacentric. Of the
Figure 3.38. *Pseudonaja textilis*, 2n = 38 (18M, 20m). Gross karyotype (upper), C-band (middle) and G-band (lower). Note the linear differentiation of the C-band pattern of the W chromosome in the central figure. Refer to text sections 3.5.17(1), and 6.6.1 and fluorescence figures, Chapter 4.
20 microchromosomes, three pairs are clearly metacentric the remaining seven pairs are acrocentric.

Pronounced C-bands in *P. textilis* are located primarily in the telomeric regions of all macrochromosomes (Fig. 3.38). A particularly large block is evident on the long arm of pair 2. The secondary constriction on pair 1 is clearly C-band positive. The Z chromosome, like the autosomes, possess prominent C-blocks on both telomeres. The W exhibits linear differentiation into lighter and darker C positive bands with a particularly dense block in the procentric region of the long arm (Fig. 3.38 and Fig. 4.1B, Chapter 4). This dense C-block reacts specifically to a variety of fluorochromes in this and other species of *Pseudonaja* (Fig. 4.3E and F, Chapter 4). G-bands are presented here for *P. textilis* (Fig. 3.38) but a comparison between species is reserved for Chapter 6.

Gillam (1979), in describing three disjunct populations of *P. textilis* from the Northern Territory, was of the opinion that *P. textilis* may represent three distinct species. I have examined specimens provided by Gillam from one such a population, as well as a specimen from Bedouri, Queensland, which represents an intermediate locality, and find all of them to possess the typical *P. textilis* karyomorph described here. As this karyomorph is distinctive and unlike that of any other *Pseudonaja* it may be valuable to examine further specimens from the isolated populations in question.
Figure 3.39. *Pseudonaja guttata* (2n = 36, 16M/20m). Gross karyotypic (above), C-band (middle) and G-band (below) preparations. Though this species possesses a diploid number of 36 with 16 macro and 20 microchromosomes, it is unlike that displayed in the "primitive" 2n = 36 karyomorph. The G-band of this and other 2n = 36 Australian elapids are compared in greater detail in Chapter 6. Refer also to text sections 3.5.17(2) and 6.6.1.
(2) *P. guttata* (2n = 36; 16M, 20m)

*P. guttata* shares the same diploid number of 36 with both *P. modesta* and *P. ingrami*, yet all three species differ considerably in gross chromosome morphology and banding details. The diploid complement of *P. guttata* is composed of 16 macro and 20 microchromosomes. While the first four pairs of macroelements appear identical to *P. textilis* in gross morphology, they differ in C-band pattern. The secondary constriction of pair 1 is C-band negative. Centric C-blocks are not evident on these four pairs and telomeric C-bands are not nearly as prominent as in *P. textilis*. Pair 5 of *P. guttata* is metacentric. Pair 6 is acrocentric with no obvious short arms, while pair 7 is acrocentric and with obvious short arms. Pair 8 is subacrocentric. Again three of the ten pairs of microchromosomes are metacentric. C-bands of macrochromosome pairs 5 to 8 indicate additional differences from *P. textilis*. Metacentric pair 5 shows a prominent centric C-block while acrocentric pairs 6 and 7 show prominent blocks both at the centromere and in the procentric regions of the long arm (Fig. 3.39). The subacrocentric pair 8 does not show an obvious centric C-band. The W appears largely C-band positive and is smaller than the Z. The G-band pattern of this species is dealt with in detail in Chapter 6.

(3) *P. modesta* (2n = 36, 16M, 20m)

*Pseudonaja modesta* possesses 16 macro and 20 microchromosomes (Fig. 3.40). The first four pairs do not appear to have altered in gross morphology from the equivalent pairs of *P. guttata* and *P. textilis*. Pairs 5 through 8, however,
Figure 3.40. *Pseudonaja modesta*, 2n = 36 (16M 20m). See text section 3.5.19(3).
differ from all other Pseudonaja species sharing the same diploid number. Pair 5 is metacentric. Pair 6 is acrocentric but displays obvious short arms. Pairs 7 and 8 are submetacentric and there are two obvious pairs of metacentric microchromosomes. This is the only species of Pseudonaja for which I do not have banding data.

(4) P. ingrami (36; 20M, 16m)

Pseudonaja ingrami is the only other Brown Snake I have examined which possesses a diploid number of 36. However, it differs from all others in possessing 20 macrochromosomes and 16 microchromosomes. All the macrochromosomes are clearly biarmed. Pairs 1 to 3 and the Z are as described above for the other Pseudonaja species. The W chromosome is subacrocentric and considerably smaller than the Z. All other macrochromosomes are metacentric or very nearly so (Fig. 3.41). It is not possible to comment on the detailed morphology of the microchromosomes from the available preparations. It does appear, however, that at least the three longest of the micro pairs are metacentric. While high quality C-banded preparations are not currently available for this species, it is still possible to rule out heterochromatic addition as being the sole explanation for the increase in number of metacentric macrochromosomes, since the large C-blocks necessary to convert two pairs of micro-elements to the macrochromosome class are not evident (Fig. 3.41). The W chromosome exhibits a lighter C-positive gap in the long arm. G-band data for P. ingrami is available for comparison in Chapter 6.
Figure 3.41 Pseudonaja ingrami (2n=36,20M/16m). This Brown Snake species again possesses a unique karyotype with 20 macrochromosomes all of which are biarmed (gross karyotypes A&B). C-banding (C) indicates very little heterochromatin on the macrochromosomes with the exception of the linearly differentiated W chromosome and the large procentric block on the eighth largest pair. The microchromosomes do not show large heterochromatic areas as are seen in some P. nuchalis forms. See text sections 3.5.17(4) and 6.6.1.
(5) *P. affinis* (2n = 34; 16M, 18m)

I have examined chromosome data from a variety of specimens of *P. affinis*. Here I present banding data from the "speckled" variety from Esperance, W.A., a colour morph which is easily recognized. A thorough survey of all colour morphs of this variable species and of *P. nuchalis* needs to be undertaken before the true significance of the variation in colour and pattern can be assessed. *P. affinis*, as described here, has a similar gross karyotype both to the insular subspecies *P. a tanneri* and to specimens of *P. nuchalis* from the extreme Southern edge of the range of that species.

In *P. affinis* the complement of 34 can be divided into 16 macro and 18 micro-elements. The first three pairs of autosomes and the Z appear identical to those described above for other species of *Pseudonaja*. In some cells of male specimen 164 I noted a despiralization at the terminal end of the long arm of pair 2, resulting in the appearance of a "satellite" type secondary constriction. This however was observed only in some of the cells. Pair 5 is metacentric, pair 6 acrocentric with evident short arms, pair 7 metacentric and pair 8 only slightly submetacentric. All microchromosomes, except the very smallest pair, are metacentric. With regard to gross morphology, the macrochromosomes of *P. affinis* appear most like those of *P. modesta*. The lack of banding data from *P. modesta*, however, prohibits any definitive conclusion to this observation.

C-banding in *P. affinis* provides a particularly interesting picture (Fig. 3.42). While only faint C-blocks are apparent at the centromeres of all the macrochromosomes,
Figure 3.42. *Pseudonaja affinis* (2n=34,16M/18m). (A&B) Note the microchromosomes are all biarmed in this species though the smallest is submetacentric. The C-banded preparation (C) indicates large C-band positive blocks in all but one pair of microchromosomes. The G-band preparation (D) should be compared with other G-band data of *Pseudonaja* species in this chapter and chapter 6. See also text sections 3.17 and 6.6.1
all but one of the micro pairs are very largely C-band positive. The single exception is a metacentric element that has only centric heterochromatin. The remainder exhibit varying amounts of euchromatin combined with large and dense C-blocks associated with the centric and procentric regions, in some instances nearly covering the entire element. G-band data for _P. affinis_ are provided here (Fig. 3.42).

(6) _P. nuchalis_ (34; 16M, 18m)

Because of the diversity of coloration and back pattern present in _P. nuchalis_ a thorough survey of this "species" is urgently needed. Specimens of _P. nuchalis_ from the southern extreme of the range resemble the karyotype described above for _P. affinis_ and have an apparent uniformity of karyotype despite showing considerable variation in colour and pattern. Further comment on this form of _P. nuchalis_ will appear in Chapter 6. The two remaining forms currently included in _P. nuchalis_ by other workers (Gillam, 1979; J. White, 1981) I chose to treat separately since they are chromosomally completely unlike the southern _P. nuchalis_ or indeed any other _Pseudonaja_ and are easily distinguishable from these and each other on the basis of morphology, colour and back patterns. Unfortunately, as the type for _P. nuchalis_ is a specimen from Port Essington, N.T., it is not clear at this time whether the name _P. nuchalis_ should be attributed to the "2n = 30 Darwin-type" which I describe below. For this reason, and because this thesis does not represent a formal publication, I refrain from giving these forms a separate species designation.

The "Black-Headed morph" of *P. nuchalis* is widely distributed. I have obtained chromosome data for specimens from the central coast of Western Australia and from both the Barkley Tablelands and Alice Springs within the Northern Territory. All of these have a diploid number of 32 but the distinction between macro and microchromosome size classes is unclear due to a gradual decrease in chromosome size. Pairs 1 to 3 and the Z appear unchanged compared with other *Pseudonaja*. The W is smaller than the Z and submeta to subacrocentric. Pair 5 is subacrocentric, pairs 6 and 7 metacentric and pair 8 telocentric (Fig. 3.43). Pairs 9 and 10 are metacentric, pair 11 telocentric and pair 12 acrocentric with distinct short arms. Of the remaining four pairs (13-16) two are metacentric while the two smallest are acrocentric.

The C-band data provided here shows the W to be largely dark heterochromatin. There is a prominent telomeric C-block on the long arm of the Z and a faint telomeric block on the long arm of pair 2. Faint centric heterochromatin is seen on pair 7 and on all the "micro" elements from pairs 9 to 16. It is slightly larger on acrocentric pair 11. It should be noted here that the microchromosomes do not display the large and intense C-positive blocks described in *P. affinis* or the subsequent morph of "*P. nuchalis*" described below. G-bands are provided for comparison here (Fig. 3.43).

(8) Sp. Nov. 2 "P. nuchalis" Darwin type (2n = 30, 16M, 14m).

The macrochromosomes of this species appear identical to those described above for *P. affinis* in both gross morphology
Figure 3.43. *Pseudonaja "nuchalis"* Black-headed morph (2n = 32, macro-micro indistinct). This morph of *P. nuchalis* differs radically from that illustrated in Figure 3.44 both in diploid number and banding details of all macroelements smaller than the Z. Note also that the micros are not largely C-band positive though they do show centromeric C-bands (below). G-band data for this species can be found in section 6.6.1. Refer also to text section 3.17(7).
Figure 3.44. "Pseudonaja nuchalis" Darwin type (male) $2n = 30$ (16M 14m). Gross karyotype (upper), C-bands (middle), and G-bands (lower). Note extensive C positive material throughout the microchromosomes seen in the central figure. Refer to text sections 3.5.17(8) and 6.6.1.
and G-banding patterns (Fig. 3.44). However it has four less micro-elements and all the microchromosomes are biarmed. Of the seven pairs of microchromosomes, three are submetacentric, while the remaining four pairs are metacentric. All appear largely heterochromatic in C-band preparations. G-bands are also available for this form.

While a more extensive survey of chromosome banding data is clearly desirable in this large and variable group of species, it is apparent from the preceding discussion that cytological data may well be of considerable importance in detecting distinct forms within species that appear, on overview, to be too variable and complex to delimit by conventional taxonomic procedures.

3.6 The Chromosomes of the Sea Snakes

Gorman (1981) has recently reviewed the published data on Sea Snake karyology and it is clear from his review that this is the elapid group for which, prior to this thesis, there was the most gross karyotypic data available. This is largely a result of the work of Singh (1972a and b, 1974). In total, Gorman reviews the chromosome data for 11 species representing 6 genera. The Sea Snakes are generally considered to be represented by over 15 genera and 55 species and their relation to terrestrial elapids is still not clear (see Chapter 1). I here present gross karyotypic data and some banding details on an additional 4 genera and 6 species. Sea Snakes vary in diploid number from 32 to 40 with some hydrophiids having undergone rearrangements of the W sex chromosome, giving rise
Chapter 1 of this thesis discusses in some detail the various classifications of the Sea Snakes. From this we can see that the species for which gross karyotypic data has been published all belong to either the laticaudine Sea Snakes or the advanced hydrophines. In the present study I have had the opportunity to examine a laticaudine Sea Snake as well as several species of the genus *Aipysurus* and *Emydocephalus* which are regarded as either a distinct but intermediate group of hydrophiids (McDowell 1967, 1972, 1974) or a true intermediate between *Laticauda* and the hydrophiids (Smith, 1926). I have also looked at *Disteria* which Voris (1977) considers "relatively primitive" and possibly divergent from the main and more recent hydrophiid stock. Burger and Natsuno (1974) have placed *Aipysurus* and *Emydocephalus* in a separate subfamily from *Disteria* (*Hydrophis*) and the other hydrophiids. So, in brief, we see most authors feel that *Aipysurus* and *Emydocephalus* appear to form some sort of intermediate between *Laticauda* and the hydrophiids.

### 3.6.1 Chromosomes of the Genus *Aipysurus*

I have examined chromosome data from three of the seven species of the genus *Aipysurus* currently recognized, namely *A. duboisi*, *A. fuscus* and *A. laevis*. All possess a diploid number of 32 consisting of 14 macrochromosomes and 18 microchromosomes (Fig. 3.45). Indeed all have very similar karyotypes. Again pairs 1 and 3 are metacentric, while pair 2 is submetacentric. The sex chromosome pair is the 4th largest
Figure 3.45. Gross karyotypes of three Aipysurus species. They are *A. duboisii*, *A. laevis*, *A. fuscus* in descending order. All have a diploid number of 32 with 14 macro- and 18 microchromosomes. Variation appears restricted to the smallest pair of macrochromosomes, pair 7. See text sections 3.6.1 and 6.6.4.
pair with the Z exhibiting a median centromere. The W chromosome in *A. laevis* is subacrocentric and only slightly larger than the Z. Pair 15 is metacentric in all three species examined, while pair 6 is subacrocentric. Pair 7 in *A. duboisi* and *A. fuscus* is metacentric, while it appears slightly submetacentric in *A. laevis*. Of the nine pairs of microchromosomes, three appear to be biarmed. All three species possess a prominent secondary constriction on one arm of chromosome 1, as is seen in many other Sea Snakes, as well as in the terrestrial Australian elapids.

### 3.6.2 Chromosomes of Emydocephalus annulatus

Gross karyotypic preparations of the highly specialized *Emydocephalus annulatus* appear essentially identical to those described above for *A. fuscus* and *A. duboisi* (compare Fig. 3.46 with Fig. 3.45). It should be noted that these genera are often placed together in the "Aipysurus" group. Unfortunately banding details are not available for *E. annulatus*.

### 3.6.3 The Chromosomes of Disteria

Of the four species once occupying this genus only two have been retained by recent taxonomists. Of these I have examined *Disteria major* and found it to share the same diploid number as described above for *Aipysurus* and *Emydocephalus*. That is $2n = 32$ with 14 macrochromosomes and 18 microchromosomes. They also share great similarity in the gross morphology of the first three pairs of autosomes in that pairs
Figure 3.46. Gross karyotype of the Sea Snake *Emydocephalus annulatus*, 2n = 32 (14M/18m). Refer to text sections 3.6.2 and 6.6.4.
1 and 3 are metacentric with an obvious secondary constriction on pair one. Pair 2 is submetacentric and there is a faint secondary constriction on the large arm appearing in some cells. The sex chromosome pair is again the 4th largest element with the Z having a median centromere and the W, of similar size, having a submedian centromere. Pairs 5 and 6 are metacentric or very nearly so, while pair 7 is acrocentric with only minute short arms. Of the nine pairs of microchromosomes at least three are biarmed (Fig. 3.47).

C-banding demonstrates centric blocks on all autosomes including the micros. The largest blocks are on pairs 3, 5, 6, 7 and the Z. Pair 2 possesses small procentric C-blocks on both arms as well as a telomeric block on the long arm. The Z chromosome possesses considerable C positive material at and near the centromere. The submetacentric W exhibits differentiation of lighter and darker C-band blocks (Figs. 3.47 and 4.1C). Chromosome pair 6 possesses faint telomeric bands on both arms. Acrocentric pair 7 has a large C-block covering the centric and procentric region perhaps involving the entire short arm (Fig. 3.47).

So we see that while there is similarity between the karyotypes of Disteria on the one hand, and those of Aipysurus and Emydocephalus on the other, they differ in centromere position on pairs 6 and 7. Until banding data is available for Aipysurus it is impossible to determine if this is simply the result of heterochromatic addition or whether it involves a more extensive rearrangement.
Figure 3.47. *Disteria major* (2n 32, 14M/18m). Gross karyotype (upper) and C-banded (lower) preparations. Note secondary constrictions on pairs 1 and 2, C-band centromeric regions and C-banded telomeric region of the long arm of pair 2. The W chromosome also shows linear differentiation of lighter and darker C positive regions. See text sections 3.6.3, 6.3.2 and 6.6.4 and Fig. 4.1.
3.6.4 Chromosomes of the Laticauda

In his review of Sea Snake karyotypes Gorman (1981) presented gross karyotypic data for 2 species of *Laticauda*, *L. colubrina* (2n = 34), *L. semifasciata* (2n = 38), and a second division karyotype from meiotic material of a third species, *L. laticauda* (n = 20, 2n = 40). The gross karyotypes of the two former species can be associated by Robertsonian rearrangement of the macrochromosomes. During the course of this study I have had access to only a single male specimen of *L. colubrina* from Tonga, Pacific Ocean. The gross karyotypic data for this species agrees with that reported by Gorman (1982) and is surprisingly similar to that described here for *Disteria major* except for the presence of an additional pair of microchromosomes in *Laticauda* (compare Fig. 3.47 with Fig. 3.48). *L. laticauda* also differs from *L. semifasciata* by the presence of another pair of additional microchromosomes bringing the diploid number in *L. laticauda* to 40.

The C-band data presented here for *L. colubrina* shows very strong centric C-bands on all macrochromosomes (Fig. 3.48) and hence differs somewhat from the condition in *Disteria*.

On the basis of similarity of gross karyotype in *Laticauda*, a general conservation of macrochromosomes in Sea Snakes and the presence of a similar (2n = 34; 14 M + 20 m) gross karyotype in the Australian Terrestrial elapid *Notechis*, Gorman (1981) has suggested that *Notechis* and *Laticauda colubrina* share a "primitive" karyotype and that this karyotype may indeed represent the ancestral condition for the elapid radiation. The validity of this concept will be judged later in this thesis in light of the additional data presented here on Sea Snakes as well as the data from the terrestrial elapids.
Figure 3.48. *Laticauda colubrina* male (2n = 34, 14M/20m).
Gross karyotype (upper) and C-band (lower) preparation. Note
the C positive centric regions. See text sections 3.6.4 and
6.6.4.
CHAPTER 4
Sex Chromosome Differentiation

4.1 Introduction

Simple sex chromosome systems fall into two basic categories involving respectively male (XY, XX) and female (ZZ, ZW) heterogamety. In vertebrates the former condition is found in most mammals, reptiles and some amphibians and fish, while the latter obtains in all birds and some reptiles, amphibians and fish. The development of such a system has always been assumed to be a secondary phenomenon. That is, cytologically differentiated sex chromosomes have arisen during the course of evolution from a progenitor pair of homologues indistinguishable in mitotic morphology and with complete pairing at meiosis. The lower vertebrates (fish, amphibians and especially reptiles) offer good affirmaitory evidence for such an assumption, since in all three groups related species are known with and without differentiated sex chromosomes.

Until recently it has also been assumed that differentiation of sex chromosomes depended on the creation of a genetically differential segment between the two progenitor homologues which permits the differential accumulation of sex factors. This, it was argued, was a progressive process involving the gradual extension of genetically differentiated segments, which do not cross over (Darlington, 1958), at the expense of the pairing segments, which do cross over. It has been argued that accompanying the development of a genetically
differential segment there has often been a reduction in the size of the Y or the W element. As such there are two obvious mechanisms which might lead to the development of differentiated sex chromosomes: (1) chiasma localization, and (2) pericentric inversion. Both of these processes lead directly to the development of pairing and differential segments. No good case of the former is known although a possible example exists in the three-toed Congo Eel, *Amphiuma tridactylum*, where the long arms of one of the longest bivalents are invariably unpaired at male meiosis and hence do not cross over (Donnelly and Sparrow, 1965). The second mechanism is known in at least three species, namely *Phyllodactylus marmoratus* (King and Rofe, 1976), *Cnemidophorus tigris* (Bull, 1978) and *Acrantophis dumereli* (Mengden and Stock, 1980). In all three cases the sex pair is undifferentiated in overall length but the centromeres of the two chromosomes are located at different sites. Added to this, in all three cases, G-banding indicates clearly that there has been a pericentric inversion.

Recently Singh et al. (1976) have suggested that in snakes, and by extension other cases too (Schmid, 1979, 1980), heterochromatinization may be the critical step leading to the initial differentiation of the sex chromosome pair and that where other morphological differences occur between sex chromosomes these are secondary consequences of the differentiation process. This argument is based on three facts: (1) in *Ptyas mucosus* (Colubridae) the Z and W are indistinguishable in length and centromere position but they are differentiated in C-band characteristics. Whereas the Z has only a small centric C-band the whole of the W is 'grey' C-band positive.
(2) Using the technique of in situ hybridization it can be shown that a specific satellite DNA, sat III, extracted from female tissues of Elaphe radiata (Colubridae) is concentrated exclusively on the C-band positive W of Ptyas mucosus. The same is true of Natrix piscator (natricine Colubridae) as well as Bungarus caeruleus and B. fasciatus (Elapidae). (3) In Python reticulatus (Boidae) and Xenopeltis unicolor, without differentiated sex chromosomes, sex chromosome specific satellite III DNA does not hybridize to any of the chromosomes.

The case of Cnemidophoris tigris (Bull, 1978) makes it clear that not all systems conform to the Singh model. Neither can the argument of Singh et al. explain all snake systems. In Acrantophis dumereli (Boidae) there is a ZW system in which the two sex chromosomes are clearly differentiated by a pericentric inversion, as confirmed by G-banding, but only a small amount of C-banding obtains in both Z and W. Interestingly this is the only known boid with differentiated sex chromosomes.

Thus what is apparent is that there is no uniform solution applicable to all sex chromosome systems. Nevertheless there are clearly good grounds for regarding heterochromatinization as one mechanism by which differentiated sex chromosomes may have evolved and it is interesting therefore, to consider what may have been involved in this process. This chapter of the thesis examines in some detail the properties of sex chromosomes in snakes and describes the types of variation to be seen in the elapid species in an attempt to resolve the cytotoxic taxonomic significance of that variation.
4.2 Sex Determination in Reptiles

Bull (1980) has recently reviewed the literature on sex determining mechanisms in reptiles and concludes that two distinct mechanisms exist here: (1) Genotypic sex determination, as indicated by the presence of recognisable sex chromosomes, is common in the squamata (lizards and snakes), but rare in turtles and crocodilians. (2) Temperature dependent sex determination is common in turtles and has been reported in two species of lizards and the American alligator.

There appears to be a clear negative correlation between temperature dependent sex determination and the presence of sex chromosomes. Bull (1980) has suggested the strong possibility that the environmentally controlled temperature dependent sex determination is either ancestral to the class Reptilia or, at the very least, is of remote ancestry in turtles and crocodilians.

Differentiated sex chromosomes have been identified in two species of turtles, and many species of snakes and lizards. The sporadic occurrence of recognizable sex chromosomes in seven families of lizards, their presence in only a minority of species in any given group, the minor degree of differentiation in many instances, and the fact that both XY and ZW systems co-exist both within the same infraorder and between infraorders has caused most authors to recognize that sex chromosome heteromorphism has been independently derived many times in lizards (Gorman, 1973; King, 1977; Bull, 1980). Indeed, examples suggesting a very recent derivation of sex chromosome heteromorphism have been presented by Bull (1978), King and Rofe (1976), and King (1977).
The situation in snakes is quite different. Many if not most snake species studied exhibit differentiated sex chromosomes. In all cases the female is the heterogametic sex. This has suggested to some authors that sex chromosome heteromorphism in snakes is derived from a single ancestral stock. The common occurrence of the heteromorphic chromosomes as the fourth largest pair in the karyotype has been interpreted to imply that all sex chromosomes in snakes may have been derived by modification of the same autosomal pair (Becak, 1964).

Preliminary G-band studies, however, indicate the Z is certainly not as conserved as is the mammalian X (Mengden and Stock, 1980; Mengden, 1981 and see Fig. 6.4, Chapt. 6).

Since the discovery of heteromorphic sex chromosomes in snakes (Kobel, 1962) they have been found to be the most variable elements in the serpent genome (Becak, 1964; Becak and Becak, 1969; Baker et al., 1972 and Singh, 1972) and are responsible for the majority of intraspecific chromosome variation published to date (see also Chapter 5). Moreover, the extent of sex chromosome differentiation found in any group tends to parallel the taxonomic position of that group. Thus, families judged to be morphologically 'primitive' on the basis of skeletal characteristics (Romer, 1956), that is most boids, do not have recognizable differentiated sex chromosomes while the most extreme forms of sex chromosome differentiation appears in the morphologically 'advanced' snakes (Viperidae and Elapidae). Colubrids occupy an intermediate position with some species in a given genus having homomorphic sex chromosomes, while other species in the same genus have heteromorphic sex chromosomes. Both Becak (1964) and Ohno (1967) have
described this differentiation process as a stepwise linear progression from a homomorphic to a heteromorphic state. In the early stages of this progression the centromere position, but not the overall size, has changed, with the most derived condition to be seen in some crotalids (Viperidae) where the W is reduced in size compared with the Z. As we shall see from the banding studies presented in the next section, the W chromosomes of the Elapidae may represent the most extreme forms of differentiation observed in Serpentes as indicated by, (1) the radical increase in the size of the W chromosome as compared to the Z, and (2) by the linear differentiation of both C-band character and differential segregation of satellite DNA as demonstrated by both fluorescence and in situ hybridization of the Bk minor satellite. In this thesis attention is directed to the 'advanced' family Elapidae though examples are occasionally provided from the 'intermediate' (Natracine, Colubridae) and 'primitive' (Boidae) families as well.

4.3 Linear Differentiation of the W chromosome in Snakes

Until recently the W chromosome of snakes, as well as that of many other vertebrates, was generally considered homogeneous in C-band character. Indeed, most studies depict the W chromosomes of snakes to be completely C-band positive throughout its length (Singh, 1976, 1980) except in cases involving the translocation of an autosomal segment, which then maintains its banding characteristics and behaviour on the W (Singh et al. 1979; Singh and Ray Chaudhuri, 1975). Even those
'intermediate' colubrids such as *Ptyas mucosus* and *Elaphe radiata* with homomorphic sex chromosomes are characterised by one member of a pair of homologues in the female karyotype which is C-band positive throughout its length. Mengden (1981), on the other hand, has recently demonstrated linear differentiation of the C-band pattern of the W chromosome in a variety of colubrid and elapid snakes as well as in bird species, and other examples are apparent in the literature. Indeed a glance at the figures in this chapter illustrates that the W chromosome in elapids is neither homogeneous in C-band character nor in satellite DNA content or base pair composition. Figure 4.1 A-C, presents C-band data for the W chromosome of fourteen species of Australian terrestrial elapids, one sea snake and three African elapids. Each chromosome shows taxon-specific linear differential staining displaying patterns of lighter and darker C-positive bands along its entire length. The lighter C-band positive regions, however, are darker than typical euchromatic areas. A comparison of these data with the G-band patterns of the W chromosomes illustrated in Figs. 4.1 (A and D) and elsewhere (in Chapter 3) demonstrates that there is no simple inverse relationships between dark C-band regions and the G negative or interbands as has been reported in the entirely heterochromatic arms of some autosomes of the boid *Sanzinia madagascarensis*. (Compare Figs. 3A and 10 in Mengden and Stock, 1980). Differences in the staining intensity of sex chromosomes after C-banding, similar to that reported here, have been seen in the Y chromosome of *Drosophila melanogaster* (Hsu, 1971), Man (Jalal et al., 1974a), the Caribou (Pathak and Stock, 1974) and
Figure 4.1. Linear differentiation of C and G-banding patterns of the W sex chromosomes of elapid snakes. (A) - C- and G-band comparisons of N.s. = Notechis scutatus and P.p. = Pseudechis porphyriacus. (B) - C-bands of the W chromosomes of a variety of Australian elapid species P.t. and Na\textsuperscript{g} show different stages of contraction in the respective specimens. (C) - C-bands of the W chromosomes of some African elapid species and a Sea Snake. (D) - G-banded W chromosomes of some Australian elapid species.

In this figure: Aa = \textit{Acanthophis antarcticus}  
A.l. = \textit{Aspidelaps lubricus}  
A.s. = \textit{Austrelaps superba}  
C.n. = \textit{Cryptophis nigrescens}  
D.c. = \textit{Drysdalia coronoides}  
D.m. = \textit{Disteria major}  
H.b. = \textit{Hoplocephalus bitorquatus}  
N.a.\textsuperscript{S} = \textit{Notechis ater serventyi}  
N.o. = \textit{Notechis scutatus}  
N.n. = \textit{Naja nigricollis}  
N.m. = \textit{Naja melanoleuca}  
P.g. = \textit{Pseudechis guttata}  
P.p. = \textit{Pseudechis porphyriacus}  
P.t. = \textit{Pseudonaja textilis}  
S.b. = \textit{Simoselaps bertholdi}  
S.s. = \textit{Suta suta}  
T.c. = \textit{Tropidechis carinatus}
some hamster species, namely Cricetus cricetus, C. griseus and Mesocricetus auratus (Vistorin et al., 1977). Jalal et al. have classified the three former cases as examples of a distinct category of C-band heterochromatin.

A similar differentiation of heterochromatin into alternating lighter and darker C positive regions is apparent in C-band preparations of the W chromosome of birds of the genus Larus published by Ryttman et al. (1979) and photographs published by Stock et al. (1974) of the W chromosomes of the ring necked dove Streptopelia risoria and the domestic pigeon Columba livia. Ryttman et al. (1979) made no reference to the phenomenon, while Stock et al. (1974) commented that, while the W chromosomes of the species they studied were darkly staining throughout, it had a distinctly darker region near the centromere. Mengden (1981) has described in some detail the clearly linearly differentiated C-band character of the W chromosome in three species of Amazon parrot and the red-tailed hawk.

All the W chromosomes of all the snake species with heteromorphic sex chromosomes studied by Singh et al. (1976, 1979, 1980) were reported as dark C positive throughout their entire length with no sign of any differential intensity in the character of C positive regions. Indeed they specifically point out that the homomorphic W of the colubrid Ptyas mucosus gave a uniform 'grey' C-band positive reaction. Though I have not had access to the same species used by Singh et al. I have examined congeneric species, namely Naja melanoleuca, N. nigricollis and N. haje, and have found them to exhibit a clear linear differentiation in the C-band character of the W. Of course since the patterns I have observed appear to be taxon
specific this might explain the discrepancy between my own observations and those of Singh et al.. However the consistent absence of any non-centric C-blocks in the autosomes and the lack of even centric C-bands in many of the preparations used by Singh et al., suggest a possible over-exposure to the alkaline treatment. This would be expected to obliterate small interstitial bands and blurr any differential staining that might be present within the W (see Schmid, 1979 for a discussion of the effects of the BaOH technique).

Comparing their own data on banding patterns in hamster species to the DNA data of Arrighi et al. (1974), Vistorin et al. (1977) have suggested that some areas of the differentially staining C-positive regions of the Y chromosome may be deficient in highly repeated DNA sequences. Further evidence of the unique structure of the intermediate 'grey' C-band material is found in the Y chromosome of the mouse Mus musculus. This chromosome shows no dark centromeric heterochromatin but is intermediate between centric heterochromatin and euchromatin with a grey appearance similar to the W of Ptyas mucosus and the intermediate blocks on the elapid W chromosome reported here (Chen and Ruddle, 1971; Pardue, 1970; Arrighi and Hsu, 1971; Hsu et al., 1971). Pardue and Gall (1970) have shown the Y chromosome of the mouse is the only element in the genome which is totally deficient in highly repetitive satellite DNA. Whether this lighter staining C-band material represents an intermediate stage in the process of heterochromatinization is not clear.

Though Singh et al. have not recognized the differential intensity of C-banding of the W chromosomes of the snake
species they studied, they did report differences between species in the composition and localization of satellite DNA on the W following in situ hybridization of the sex specific satellite III from Elaphe radiata and the Bk minor satellite from Bungarus fasciatus. While the W chromosome of all species studied appeared completely C-positive and most labelled uniformly over the entire length with either or both sat III or Bk minor, there were several important exceptions:

(1) *Elaphe radiata*, with its homomorphic, but entirely C-band positive, W chromosome appears to label only in the intercalary region of the long arm when Bk minor sat is hybridized to it. In this same species it is claimed that its own sat III localizes exclusively to the telomeric regions (Singh et al., 1980) though the figure offered in support of this claim suggests that label is distributed over most of both arms excluding only the immediate centromere regions.

(2) In both *Naja n. naja* and *N. n. oxiana* Bk minor sat is concentrated on the long arm of the subtelocentric W chromosome leaving the minute short arm unlabelled, though in both species the entire W chromosome is said to be C-band positive.

(3) In *Bungarus walli* (published as *B. walliall* by Singh et al., 1980), where the W chromosome is considerably larger than the Z, sat III and Bk minor are both restricted to the long arm of the W. Singh et al. have suggested that the other arm contains additional sequences as yet unidentified and these account for the apparent enlargement of the W relative to the Z.
On the above evidence, it is clear that there are areas in the W chromosomes of snakes where neither sat III or Bk minor are present and other areas where they may be interspersed.

Some of the members of the 'advanced' family Elapidae possess exceptionally large W chromosomes. Thus in *Pseudechis porphyriacus* (Fig. 4.1a) the W is as large as the largest autosome and in most species the W is at least as large as the Z. Such W chromosomes would be especially interesting material in which to examine the composition of the different C-band regions. To this end, selected species were analysed following fluorochrome/counterstain treatments, which are thought to provide some indication of predominant base pair composition (Schweizer, 1981). Additionally *Pseudechis porphyriacus* was studied following in situ hybridization of the Bk minor satellite. From the in situ data it appears the W chromosome of *P. porphyriacus* shows a distinct gap in the labelling pattern. Thus whereas the centromere region itself and the majority of both arms are densely labelled there is a distinct non-labelled gap on either side of the centromere (Fig. 4.2E). This is reminiscent of the pattern of dark C positive areas on the W chromosome of this species illustrated in Fig. 4.1A. Here the majority of both arms and the centromere and the area near it on the long arm, are dark C positive, while there is an interstitial gap of lighter C positive material on each arm proximal to the centromeric blocks.

Since in situ hybridization may not be sufficiently sensitive to distinguish between short adjacent regions with and without satellite DNA, it would be logical to expand the study to examine species with distinct small dark C positive blocks,
Figure 4.2. *In situ* hybridization of the Bk minor satellite of *Bungarus fasciatus* onto the chromosomes of the elapid *Pseudechis porphyriacus* (A, C, D, E and F). This satellite is thought to be sex specific in snakes (Singh *et al*., 1976, 1980). Note the correlation between label areas of the W chromosome and darker C-band positive areas of this species seen in Figure 4.1. Note Figure 4.2B is a boid.
such as *Pseudonaja*, or large blocks localized on a single arm, as in *Suta* (Fig. 4.1B).

The data from fluorescence studies also offers some insight into the distinctive nature of these dark C-positive regions on the W chromosome, though the base pair specificity of some of the fluorochromes remains to be demonstrated. Many authors have suggested that by combining a fluorochrome with a complementary counterstain, fluorescent banding can be greatly enhanced. In the present study I have used the protocol of Schweizer (1981) in combining the GC-specific primary stain, *Chromomycin A3* with AT-specific counterstain *distamycin A*. I have also used a similar technique involving treatment with AT-specific primary stain, DAPI, followed by the GC-specific counterstain *actinomycin D*. Since the binding capabilities of these dyes have finite spatial limitations, I have paid attention only to those cases involving a radical difference in fluorescence and which I assume to depict high concentrations of distinctive material binding to the fluorochromes. The degree to which these fluorochrome combinations identify specific base pair sequences is irrelevant since I use the data simply to compare with the distinctive C-band quality of the dark regions. It should be noted that the DAPI bands have been used to differentiate distinctive regions of the human Y chromosome which the reader will recall represents one of the clearest cases of linear differentiation of C-band pattern found in a mammal. It should also be borne in mind that a strong DAPI negative reaction may indicate a GC-rich area. Good confirmation of this reverse relationship is seen in comparing Figs. 4.3A and B. Here the secondary constriction
Figure 4.3. DAPI and chromomycin fluorescence in elapid snakes. Arrows indicate W chromosomes in each spread. (A) - *Tropidechis carinatus*. (B) - *Notechis occidentalis* (C and D) - *Hemiaspis signata* (E and F) - *Pseudonaja "nuchalis"*, black-headed morph. Compare these to C-band data for these species in Chapter 3 and Figure 4.1, and see text, section 4.3.
in chromosome 1 is distinctively DAPI negative, as is the case in most Australian elapids, while many of the microchromosomes are DAPI dull. In Fig. 4.3B we see this C-band positive secondary constriction is intensely chromomycin A3 positive, as are the C-band positive telomeric regions of the autosomes of this species. Clearly there exist species-specific variation in DAPI staining regions within the W chromosome. Perhaps the best correlations between dark C-positive regions and distinctive florescent behaviour is seen in _Pseudonaja_ (compare C-bands in Fig. 4.1B and Fig. 3.38 in Chapter 3 with florescence bands in Fig. 4.3E and F) and _Suta_ (compare C-band in Fig. 4.1B with DAPI bands in Fig. 4.4A). Where even minute portions of the W exhibit fluorescence the regions in question are obvious in interphase cells (Figs. 4.3E, 4.4A and E). The only other study utilizing fluorescence to examine W chromosomes in snakes is that of Becak and Becak (1972). Here quinacrine dihydrochloride was used to demonstrate the presence of a W chromatin body in the interphase cells of two species of colubrids (_Clelia_) and a viperid (_Bothrops_). Such a body was not present in the 'primitive' boid (_Boa_).

The only species of the genus _Naja_ for which a female was studied using fluorescence techniques displayed a small but distinctive DAPI band on the W chromosome (Fig. 4.4B, D and E). Due to the effect of the _in situ_ hybridization technique on chromosome morphology, coupled with the large grain size of the label, it is not possible to determine whether this DAPI band corresponds to the unlabelled area in the Bk minor studies of _Naja naja_ and _N. oxiana_ (Singh _et al._, 1980). Thus although Singh _et al._ conclude that the minute short arm of the W is unlabelled, this is based purely on conjecture.
Figure 4.4. DAPI fluorescence of elapid snakes. Arrows indicate sex chromosomes at metaphase and the resultant chromatin body at interphase. Note the linear differentiation of the W chromosome in each case. (A) - *Suta* *suta* compare with C-band Figure 4.1. (B, D and E) - *Naja* *haje*. (C) - *Unechis* *dwyeri* compare with C-bands Figure 3.23. See also text section 4.3.
Before any further meaningful correlation between satellite regions and C-band or fluorescence behaviour can be attempted, it is necessary to apply the in situ technique to a greater variety of elapids including *N. haje* and *Suta*, or else to apply these fluorescence and banding techniques to those species which Singh *et al.* (1980) have found to exhibit satellite localization. Whatever association exists between the types of satellite DNA and the categories of C-bands, it is clear that the fluorescence data have demonstrated the distinctive nature of the light and dark C-bands and argues against any simplistic concept of homogeneity in the process of sex chromosome heterochromatization.

Additionally there may be a need to distinguish two categories of heterochromatinization in relation to sex chromosome differentiation:

(1) Primary heterochromatinization where the first step in the differentiation of the sex pair actually depends on the heterochromatinization event, though this does not preclude subsequent structural changes.

(2) Secondary heterochromatinization where heterochromatin changes follow from, rather than initially determine, the differentiation of the sex pair which would depend either on chiasma localization or else on primary structural change.

As far as primary heterochromatinization is concerned it is difficult to imagine how such a differential molecular transformation could have taken place between what must have
initially been homologous structures. Neither is it obvious how the presumed silencing of a whole chromosome has been compensated for in a developmental sense. Singh et al. (1976, 1980) have suggested that amplification and distribution of sex specific satellites has involved saltation coupled with multiple internal rearrangements which they assume to be inversions. If this is genuinely the case, the inversions in question must have been paracentric because there is no change in the gross morphology between the sex chromosome of Ptyas mucosus and Elaphe radiata. Since the W chromosome of snakes has been shown to G-band satisfactorily, (Fig. 4.1 D) one test for the inversion distribution model of Singh et al. might be to compare the G-band pattern of the Z and W chromosomes in species where they are homomorphic yet differentiated in respect to C-bands and satellite.

King (1980) has drawn attention to the possibility of transforming euchromatin into heterochromatin without any accompanying structural changes. His argument is based on comparisons of the C-banding characteristics between the autosomes of closely related species of frogs. At least one case is on record within a species, however, where a known autosomal polymorphism is involved in which one homologue is dramatically different in C-band character from its partner (Bianchi and Ayers, 1971). A similar situation has been reported for chromosome pair six in females of a population of the frog Rana clamitans (Mengden, 1981). Additional specimens from this and other populations need to be examined to determine if this sex linked polymorphism represents a genuine sex determining system. Interestingly the only other anurans
with differentiated ZW chromosomes are also ranids. These are *P.ICEPHALUS ADSPERSUS* and *P. DELANDII*, both of which show an increase in heterochromatic content in the W chromosomes. In P. adspersus the increase is coupled with a radical reduction in size of the W whereas in the other species, it is accompanied by a pericentric inversion but no size alteration. Schmid (1980), who first described these cases, follows Singh et al. (1980) in assuming that the structural changes concerned are secondary to the heterochromatinization process though there is no compelling evidence to refute or confirm such an assumption. This case clearly highlights the difficulty of distinguishing between primary and secondary heterochromatinization.

As far as secondary heterochromatinization is concerned, there is only one suggestive example so far known. Saez (1963), has argued that progressive heterochromatinization of the Y chromosome has occurred in some orthopteran neo XY systems, a suggestion that has been supported by White (1973). Their arguments, however, are based on a comparison between largely unrelated species. There is certainly good evidence from *CNEMIDOPHORIS TIGRIS* that C-band material can be lost following a pericentric inversion leading to sex chromosome differentiation (Bull, 1978). The same appears to have been the case in the evolution of the neo XY system of the orthopteran *STENOCATANTOPS ANGUSTIFRONS* (King and John, 1980). Thus secondary deheterochromatinization may also occur in the evolution of sex chromosome systems.
4.4 Multiple Sex Chromosome Systems in Snakes

Only one species of Asian terrestrial elapid (*Bungarus caeruleus*) and four species of hydrophid sea snakes are known to possess a multiple sex chromosome system. The consequent change in diploid number in these cases results from one of two distinct types of chromosome rearrangement: (1) fusion of an autosome to the W chromosome giving rise to a reduction by one in the diploid number of the female; (2) fission of the W chromosome resulting in an increase of one in the diploid number of the female. Singh *et al.* (1970) were the first to describe a W to autosome fusion in *Bungarus caeruleus* giving rise to a $Z_1Z_1Z_2Z_2/Z_1Z_2W$ system. Here it results in a diploid number of 43 in females and 44 in males. The autosome involved in this case is a macrochromosome and it maintains its autosomal replication pattern so that it is readily distinguishable from the late replicating portion of the W (Ray-Chaudhuri and Singh, 1972). This condition was later shown to be one of four sex chromosome variants present in this species. Thus Singh *et al.* (1979) have described a fusion involving the W chromosome with a microchromosome in some populations of this species. Other specimens from this and different localities show a fission of the W chromosome resulting in a diploid number of 45 in females. A fourth class possesses the typical ZW configuration found in other members of the genus *Bungarus*.

In the elapids studied in this thesis a $Z_1Z_1Z_2Z_2/Z_1Z_2W$ system has been observed in two of the three species of the genus *Hoplocephalus*, which is among the most specialized of all Australian elapid genera since it is arboreal. Here too the
autosome involved in the fusion is a microchromosome. Despite the small number of individuals analysed the occurrence of this condition in two of the three species of the genus suggests that it is not a polymorphic/polytypic condition in any particular species as is evidently the case in Bungarus caeruleus. Due to the minute size of the element involved in Hoplocephalus even C- and G-banding makes it impossible to demonstrate unequivocally that the same microchromosome is involved.

The second type of rearrangement resulting in multiple sex chromosomes in snakes, is a fission of the W chromosome giving rise to a ZZ/ZW₁W₂ system, as described above in one of the populations of Bungarus caeruleus. Such a fission has been reported in four species of hydrophid sea snakes, namely Enhydrina schistosa, Hydrophis fasciatus, H. cyanocinctus and Microcephalus (Hydrophis) gracilis (Singh, 1972a and b). Though sample sizes were small and males were not examined for the last three of the species, Singh (1972a and b) has suggested that these changes characterize each species. In H. fasciatus and M. (H) gracilis the two W chromosomes are both biarmed which suggests they have undergone further differentiation since the fission event.

In the Australian terrestrial elapids reported in this thesis a fission of the W chromosome and a concomitant ZZ/ZW₁W₂ system is present in some populations of Cryptophis nigrescens. Because this species exhibits an elaborate degree of intraspecific polymorphism both in sex chromosome and autosome morphology, it is discussed in detail in the next chapter.

From these few known examples it is clear that multiple
sex chromosome systems have evolved on more than one occasion within the elapids and may be either species-specific, polytypic or polymorphic. This lability of sex chromosome morphology and composition implies that they are not likely to be of much value as markers of phylogenetic differentiation.
5.1 Intraspecific Chromosome Variation in Snakes

In the published cytotoxic studies of reptiles there is an underlying assumption that the few specimens examined represent the species norm. The value of fixed differences in chromosome constitution between species as a phylogenetic tool depends on the validity of this assumption and in many cases the data as it stands is clearly not adequate to rigorously test that assumption.

While the reported incidence of intraspecific variation in snake chromosomes is exceedingly rare, this too undoubtedly reflects the small sample sizes which have been examined. Nevertheless, where there is evidence of intraspecific variation it has always involved the sex chromosomes. Such a situation has been described within populations of the natricine colubrids Thamnophis marcianus and Storeria dekayi (Baker et al., 1972) where both centromere position and W chromosome length are involved. De Smet (1978) has invoked a sex chromosome polymorphism to explain the discrepancy between his data on centromere position in the W chromosome of Elaphe longissima and that of Kobel (1967) though this is open to question.

One of the best documented cases of complex intraspecific sex chromosome variation is that discussed in the previous chapter for the elapid snake Bungarus caeruleus (Singh et al., 1979). Singh (1972) has also found an intraspecific difference in the gross morphology of the W chromosome of Naja naja. This difference however characterizes subspecies and is therefore polytypic not polymorphic.

No well documented example of autosomal intraspecific
variation in snakes has been published to date. A most confusing situation occurs in the natricine *Xenochropus* (Amphiesima) *Natrix* *piscator*. Here Dutt (1970) has reported two widely separated populations, one possessing 36 chromosomes with 16 macrochromosomes and 20 microchromosomes, and the other having 38 chromosomes with 10 macrochromosomes and 28 microchromosomes. She found consistent differences in size and color in specimens from these two populations and hence suggested subspecific differences may be responsible for this variation. Singh *et al.* (1968) initially reported a diploid number of 40 (16 M/20m) for this species but later (Singh *et al.*, 1970 and Singh, 1972) raised the count to 42 (18M/24m). De Smet (1978) has subsequently confirmed the diploid number of 42 for the species but reports it to have 22 macro and 20 microchromosomes. As Dutt's work consisted of squash preparations, which yielded highly variable counts within specimens, Singh (1972) disregarded her suggestion of intraspecific variation. From his own data, however, he states that two types of cells containing 40 and 42 chromosomes are present in the same individual with the difference being attributed to one pair of microchromosomes. Indeed in 2 out of 40 individuals studied the "false" diploid count of 40 was found in 60% of the cells. In the remaining 38 specimens the "majority of cells had 48 chromosomes". The simplest explanation for this variation is that it is not real but arises from the quality of the preparations used. Rossman and Eberle (1977) reported yet another diploid count for the species describing it as having 2n = 44 with 16 macro and 28 micro-chromosomes. These authors, however, provided no figure and did not make reference
to any of the previous reports.

The only other example of intraspecific variation in diploid number that I am aware of is in the elapid *Micrurus nigrocinctus* reported by Gutierrez and Bolanos (1979). Here two karyotypic populations correspond to two of the three subspecies known. Thus *M. n. nigrocinctus* has a diploid number of 26 with 16 macro and 10 microchromosomes, while *M. n. mosquitensis* possess 30 chromosomes with 16 macro and 14 microchromosomes. The populations are clearly subspecifically differentiated.

5.2 **Intraspecific Variation in Australian Elapid Snakes**

In the present study of elapid snake chromosomes, several clear examples of intraspecific chromosome variation exist. In the tiger snake *Notechis*, where differences in C-band and fluorescence character of the W chromosome have already been demonstrated (see Chapter 4, Fig. 4.1, 4.3) the variation is clearly polytypic. Within the Brown Snakes currently recognized as monotypic and identified by the specific name *Pseudonaja nuchalis*, there exist at least three karyomorphs which are distinguishable both in diploid number and banding details (see Chapter 3). Specimens of these karyomorphs are also distinguishable by morphology and color pattern. Gillam (1979) has recognized a wide variety of back pattern morphs within this taxon and the species is clearly composite (Cogger, 1975, 1979). I am of the opinion that these karyomorphs represent distinct species and will discuss them in greater
detail in Chapter 6. From these two examples, and the reports on *Naja* and *Micrurus* mentioned above, we see that some cases of intraspecific chromosome variation are likely to reflect polytypic rather than polymorphic variation, at least at the chromosome level. This may also be the case in the Death Adder (genus *Acanthophis*).

While most of the elapid species which I have studied are consistent in the details of chromosome banding throughout all the specimens examined, this is not the case in the Death Adder. Within Australia three species are currently recognized: *A. antarcticus* has an extensive range in Eastern and South Western Australia, excluding the arid central deserts and Southern Victoria and Tasmania. This species varies considerably in gross morphology, coloration and size and may be composite. *Acanthophis pyrrhus* is a red, desert adapted, form restricted to the central and Western deserts. Storr (1981) recognizes a third species, inhabiting the Kimberley region of W.A. and the Northern portion of the Northern Territory. For this species he has resurrected the name *A. praelongus*. All three species have the same diploid number of 36 with 16 macro and 20 microchromosomes and in all cases the smallest three pairs of macrochromosomes are acrocentric. The various forms differ, however, in the number, location, and composition of secondary constrictions present in the karyotype. Thus, specimens of *A. antarcticus* from S.W. Australia possess only the secondary constriction on the long arm of the largest autosome which is to be seen in most Australian elapids (specimen 206, Fig. 5.1). Specimens from other southern localities have an additional secondary constriction in the procentric region of
Figure 5.1. Gross karyotypes of *Acanthophis antarcticus*, *A. pyrrhus* and *A. antarcticus*? Adelaide River N.T., in descending order. Note *A. antarcticus* exhibits secondary constrictions on both homologues of pair 1 as is the case in most Australian elapid species, while *A. pyrrhus* has additional secondary constrictions on pair 2. The Adelaide River specimen is heteromorphic for secondary constrictions on pairs 1 and 2 and homomorphic for a constriction on pair 6. For detailed discussion of the variation in *Acanthophis* see text sections 3.5.8, 5.2 and 6.6.1.
pair 6. *A. pyrrhus*, on the other hand, possesses a secondary constriction in the procentric region of the long arm of pair 2 in addition to that on pair 1 (Fig. 5.1). *A. praelongus* has prominent secondary constrictions on pairs 1, 2 and 6. Specimens currently recognized as *A. antarcticus* from the Barkley Tablelands of the Northern Territory, unlike their conspecifics to the south, exhibit secondary constrictions in the procentric region of the long arm of pair 2. These specimens are also distinctive in external morphology, body proportions and coloration from the other representatives of *A. antarcticus* as well as from other species of *Acanthophis*. One specimen from Adelaide River, N.T., which appeared intermediate in coloration and gross morphology, proved to be heteromorphic for the secondary constrictions on pairs 1 and 2 but possessed a secondary constriction on both homologues of pair 6 (Fig. 5.1).

These several forms were therefore analysed with C-, G- and N-banding in addition to the fluorescence techniques and in situ hybridization of 18S + 28S rDNA. These data are summarised in Fig. 5.2. As is clear from the figures, the various types also differ in the banding and fluorescence character of the W chromosome. The secondary constriction on pair 1 is C-band positive only in some southern specimens of *A. antarcticus*. It is, however, N-band positive in *A. antarcticus* and DAPI/negative in all the forms studied. In situ hybridization indicates that this secondary constriction is the only site of 18S + 28S RNA genes. It is heteromorphic for in situ labelling in the Adelaide River specimen where it also appears heteromorphic in both the gross karyotype and
following DAPI fluorescence. Where present, the secondary constriction in the procentric region of pair 2 is C-band positive and is heteromorphic for this condition and for the N-band reaction in the Adelaide River animal. It also coincides with a peculiarly dense G-band positive area where it occurs in *A. pyrrhus* as well as in the heteromorphic condition in the Adelaide River specimen. A similar dense G-band area is seen on the W chromosome and is also associated with the secondary constriction on pair 6 of these specimens. In DAPI fluorescence the secondary constriction on pair 2 varies considerably being DAPI positive in the heteromorphic state in the Adelaide River specimen (Fig. 5.6) but, DAPI negative in *A. pyrrhus* and undifferentiated in southern *A. antarcticus*. The W chromosomes also vary in these forms in C-, G- and N-bands and fluorescence as summarised in Figs. 5.2, 5.4 and 5.5. The secondary constriction on pair 6 is C-band and N-band positive, where it occurs, but is densely G-band positive and DAPI fluorescent only in the Adelaide River specimens. From a composite analysis of these data we can see that each form displays a distinctive pattern in the location and composition of the secondary constriction. Likewise, though a particular constriction may appear identical in gross morphology and C-band character between species, it can be demonstrated to be of different composition when a variety of techniques are applied. These data, along with those presented in the previous chapter, demonstrate that, in snakes at least, the N-banding technique does not consistently demonstrate the location of the 18 + 28S RNA genes. Likewise it is apparent that when attempting to use nucleolar organizer regions as
Figure 5.2. A schematic summary of banding data obtained for pairs 1, 2, b and the W chromosome of the Death Adders, Acanthophis. Note that all four chromosomes show specific patterns in regard to the combination of banding procedures employed to elucidate the homologies of the secondary constrictions observed. Only the secondary constriction on pair 1 however labels with the 18 + 28s rRNA probe. See text section 5.2 for detailed discussion.
Figure 5.3. C-banded preparation of the macrochromosomes of *Acanthophis pyrrhus* (upper). Arrows indicate prominent C-bands in the procentric regions of pairs 2 and 6 which may coincide with secondary constrictions. G-banded preparations of *Acanthophis pyrrhus* (middle left) *A. antarcticus* Adelaide River specimen (middle right) and *Acanthophis antarcticus* (bottom). Arrows indicate prominent G positive bands associated with C-bands and secondary constrictions. Note these bands are heteromorphic in the Adelaide River specimen which is also heteromorphic for the secondary constriction (see Fig. 5.1 and 5.2).
Figure 5.4. G-band comparisons between the Death Adders, genus Acanthophis. In this figure:

A.d.R. = Acanthophis antarcticus Adelaide River specimen.
A.p.y. = A. pyrrhus
A.a. = Typical southern form of A. antarcticus

Note: G-bands are similar except for the prominent bands associated with secondary constrictions and of course, the W chromosome. See text sections 3.5.8, 5.2 and 6.6.1.
Figure 5.5  N-banded preparations of *Acanthophis antarcticus* (A) and *A. antarcticus* Adelaide River N.T. specimen B. Note that in both instances there is linear differentiation of the W chromosome. This suggests that the N-band technique may not be specific for NOR's in snakes. Comparing these data to the *in situ* hybridization data (Figure 6.2) supports this conclusion. See Figure 5.2 for a summary of data characterizing NOR's in *Acanthophis*.
Figure 5.6. DAPI fluorescence in elapid snakes. (A and B) - Acanthophis Adelaide River, arrows indicate W chromosome (large arrow) and sites of secondary constrictions. Note: secondary constrictions on pair 1 are DAPI negative, while those on pair 2 are heteromorphic DAPI positive and those on pair 6 homomorphic DAPI positive. See Figures 5.2 for summary and refer to text section 5.2. (C) - Heamachatus heamachatus male. Note prominent centromeric C blocks are DAPI positive while the presumed Z has a DAPI negative area. Compare with C-band Figure 3.4. (D) - Ophiophagus hannah male again some centromeric C-blocks are DAPI positive. Compare with C-bands Figure 3.9.
Figure 5.7. Map illustrating location and chromosome composition of 4 populations of *Cryptophis nigrescens* sampled. Note populations are heterogeneous with respect to the occurrence of both sex chromosome and autosome polymorphisms. Refer to text section 5.2.
fixed differences between groups, such as has been done in anurans (Early, 1971; Schmid, 1978 and King, 1981), it is wise to characterise these regions by a variety of methods in addition to C-banding and Ag-staining.

If the variation in secondary constrictions in *Acanthophis* is indeed a marker of taxonomic distinctiveness, as are the subspecies-specific differences in *Naja naja* and *Micrurus nigrocinctus*, there exists only one unequivocal case of autosomal polymorphism in snakes. This occurs in the chromosomally highly variable Australian Small Eyed Snake *Cryptophis nigrescens* which I will deal with next. This species is distributed along the coastal ranges of eastern Australia from Cape York through Victoria and has been reported to vary in adult size and ventral coloration. Pollitt (1981) has analysed the venom of specimens throughout the species range utilizing two directional horizontal polyacrylamide gel electrophoresis. He finds that "venom had a pattern characteristic of the species and that only insignificant individual variations occurred". From the chromosome data which I will now present, it is apparent that the species exhibits complex polymorphisms in sex chromosome morphology and number as well as in autosomal morphology and heterochromatin content. All males possess a diploid number of 40, while females may have 40 or 41 chromosomes depending on whether they have a conventional ZW sex chromosome system or a multiple system of the ZZ/ZW₁W₂ type. Moreover, at least four distinct variants of W chromosome morphology exist. The number of chromosomes in the macro and microchromosome classes also varies radically between specimens and includes the following categories: 20M/20m
(specimens 321, 288, 278, 279), 21M/19m (284), 22M/18m (201, 198), 23M/18m (261). C-band data indicates that some of these differences in micro and macrochromosome number are clearly the result of heterochromatin addition to microchromosome elements (see specimens 279, 198, 284, 201). However, discrepancies in the total amount of heterochromatin on a variety of chromosomes make it clear that heterochromatin addition cannot explain all changes (compare Figs. 3.19, 5.14). Wherever large heterochromatic blocks appear in the autosomes they are DAPI positive in fluorescence and N-band positive (Fig. 5.13). In situ hybridization with a 18 + 28S rRNA probe labels only the secondary constriction on the largest autosome, demonstrating again that N-banding is not specific for nucleolar organiser regions in snakes, since DAPI positive dark C-band segments of the W chromosome of this and other species N-band but hybridization is restricted to secondary constrictions. These large autosomal heterochromatic blocks do not result in large G-band negative regions (compare Figs. 5.10 and 5.14).

Figure 5.7 summarises the data on the location of polymorphic variants. From this map and the banding data I believe the basic karyomorph for the species has a diploid number of 40, with 20 macrochromosomes and 20 microchromosomes, and an acrocentric W chromosome only slightly larger than the submetacentric Z. Such a karyomorph is found in a majority of specimens in all but one population sampled and could easily be derived from the only other species known in the genus, Cryptophis pallidiceps, (2n = 36) by a Robertsonian rearrangement involving the third largest chromosome pair of that species. Though banding data is not available for
C. pallidiceps a similar pair 3 appears to have been conserved throughout most Australian elapid species. Banding details indicate that, in place of this metacentric pair 3, C. nigrescens possesses two pairs of acrocentric chromosomes (see G-band Fig. 3.19).

Surprisingly specimens with the basic 2n = 40 karyomorph (specimen 321, Fig. 5.8 and 288 Chapter 3) possess a large amount of heterochromatin on a majority of the acrocentric macrochromosomes. An examination of prometaphase cells indicates that these apparently single blocks are in fact interspersed with euchromatin and are not G-band negative (Figs. 5.8 and 5.10, 321, Figs. 3.19, 5.14), Male 284 (Fig. 5.9), with a configuration of 21 macrochromosomes and 19 microchromosomes, can be derived from the basic condition by heterochromatic addition to a microchromosome and an examination of Figures 5.9 and 5.10 indicates that this is the case. G-bands (Fig. 5.) demonstrate the presence of a novel macrochromosome with an abnormal G-band pattern. Unfortunately the size of the microchromosomes precludes a precise identification of its homologue.

Heterochromatin addition also appears to be responsible for heteromorphism in several pairs of small acrocentrics. An interesting example is seen in specimens 278 and 279 which are litter mates. Both possess a 20 macro + 20 microchromosome complement, yet the female displays a W chromosome that is much larger than the submetacentric Z. The male (279) on the other hand, has an obvious heteromorphism in the smallest pair of macrochromosomes and C-band data clearly indicates heterochromatin addition (Fig. 5.11). In specimen 261 (23M/18m;
Figure 5.8. Gross karyotypic (upper) and C-band (lower) data for a "typical" female specimen of *Cryptophis nigrescens* (specimen 321, 2n = 40, 20M/20m). Despite a gross karyotype, apparently only slightly altered from that of *C. pallidecephs* and *U. carpentariae* (centric fission/fusions) C-band details indicate a radical difference in the heterochromatin content of the acrocentric (compare with Fig. 3.25). See text of section 3.5.7, 5.2 and 6.6.1.
Figure 5.9. Gross karyotypic and C-band data on Cryptophis nigrescens male specimen 284 (2n = 40, 21M/19m) illustrating the apparent conversion of a microelement to 9 macrochromosome category by heterochromatic addition. See text sections 3.5.7, 5.2, 6.2.2 and 6.6.1.
Figure 5.10. C-band karyotype of macroelements of *Cryptophis nigrescens* specimen 284 (2n = 40 21M/19m). Note heterochromatic addition to a microelement giving rise to a heterozygous pair 11. Also, there are extensive C-band positive blocks on autosomal pairs 4, 5, 8 and 9. See text sections 3.5.7, 5.2 and 6.6.1.
Figure 5.11. (Above) - Gross karyotype and C-bands of the sex chromosomes of Cryptophis nigrescens, female specimen 278 (2n = 40, 20M/20m) illustrating one of four W sex chromosome variants found in this species. (Below) - Gross karyotype and C-band data for the sex chromosome pair and heteromorphic autosomal pair 10 of C. nigrescens, male specimen 279. The C-band data indicate that the heteromorphism of pair 10 is due to the addition of a large block of heterochromatin. Note, these two specimens are litter mates. See text section 3.5.7, 5.2 and 6.6.1.
Figure 5.12. Small eyed Snake *Cryptophis nigrescens* (2n = 41; 23M, 18m), female specimen 261. Gross karyotype (upper) and C-banded (lower) preparations. Note multiple sex chromosome system and large heterochromatic regions on autosomes. Refer to section 5.2 of the text and map Figure 5.7.
Figure 5.13. Comparison of N and C-bands in Cryptophis nigrescens (A and B) indicate both large and small N-positive regions, while (C and D) indicate variation in heterochromatin content. Comparing A to Figures 5.8, 5.9, and 5.10, we see a close correlation between the large C positive blocks on the acrocentric macroelements and the large N-band regions. These regions do not however label with the 18 + 28s RNA probe (see Fig. 6.2). Faint N bands can be seen at the secondary constriction on pair 1 which does label with the 18 + 28s rRNA probe. See text sections 5.2 and 6.3.1.
Fig. 5.12) there is an additional pair of small acrocentrics, yet this pair, as well as pairs 6, 8, and 9, appear to have relatively less heterochromatin than the equivalent pairs in other specimens (compare with 284, Fig. 5.9). Similar discrepancies in heterochromatin content are suggested from the comparison of C- and N-banding in Fig. 5.13. Here specimen 298 (21M/19m) appears to have a great deal more N positive heterochromatin than does 291 (21M/20m). Male specimen 198 (Fig. 5.14) exhibits a 22 macrochromosome 18 microchromosome configuration complementary to female 201 which has yet another sex chromosome heteromorphism and both specimens from the same locality exhibit obvious heteromorphism in the smallest macrochromosome.

Finally, it is apparent from the map (Fig. 5.7) that the sex chromosome polymorphism must be independent of the autosomal polymorphisms since female individuals with the 20M 20m complement possess three distinct W chromosomes while multiple sex chromosomes were observed in two distinct autosomal backgrounds.
Figure 5.14. (A) *Cryptophis pallidiceps* (2n=36, 20M/16m) female gross karyotypic preparation. Note that the macro and micro-chromosome categories are ill defined. (B) *Cryptophis nigrescens* male specimen number 284 (2n = 40, 21M/19m) G-band preparation. Note the large C-band regions in the acrocentric macrochromosomes of this specimen (compare with figure 5.10) are G-band positive.
Figure 5.15. Gross karyotypic data of male (198) and female (201) specimens of *C. nigrescens* possessing a diploid number of 40 but composed of 22 macro and 18 microelements. The heteromorphism, in both specimens, of novel macrochromosome pair 11 suggests heterochromatic addition. C-banding of the W sex chromosome provided in the lower figure illustrates a distinctive variant of this chromosome. See text sections 5.2 and 6.6.1.
CHAPTER 6

The differentiation of the various species of the genus A. vaillantii has been discussed in previous chapters and the results will be given in the present chapter. An attempt has been made to correlate the characteristics of these species with their ecological and geographical distribution.
6.1 Preamble

The chromosome studies collated in the previous chapters of this thesis represent the first serious attempt to obtain comprehensive cytological data for a majority of extant species within a single large family of snakes, the Elapidae. This substantial data bank provides the opportunity for analyses of two kinds:

(1) a consideration of the mechanisms of chromosome change which have been involved in producing the variation evident in present day forms, and

(2) the collection of species into karyomorph groups on the basis of shared fixed differences in an attempt to provide information on the relationships within and between these groups.

The first approach, the identification of the principal mechanisms of chromosome change which have occurred within the elapids, was the underlying theme of the descriptive presentation reported in Chapter 3. Additionally an entire section of the thesis was devoted to the specific mechanisms involved in sex chromosome differentiation (Chapter 4, and Sections 5.1 and 5.2 of Chapter 5 inter- and intraspecific variation). After summarizing the conclusions of these chapters, I will discuss in some detail the problem of microchromosome
evolution, and then turn to the categories of structural chromosome change which appear to have played a role in elapid evolution.

6.2 Changes Associated with Heterochromatin

6.2.1 Sex Chromosome Differentiation

It has become apparent for the first time from the data presented in this thesis that the differentiation of the sex chromosome pair in snakes can be the result of two distinct avenues of change, namely, (1) modification of the W chromosome and (2) modification of the Z chromosome.

(1) W chromosome differentiation

While the female is the heterogametic sex in all snakes for which sex chromosome data is available, it has been clear for some time that species exist which exhibit both homomorphic and heteromorphic sex chromosomes in terms of gross chromosomal morphology.

Prior to this thesis few studies have utilized banding and fluorescence techniques to determine the mechanisms involved in producing heteromorphy. As a result of the banding work described in Chapter 4, the W chromosome of many snakes can now be seen to exhibit a linear differentiation of lighter and darker C positive regions along the length of the chromosome, and the same appears to be true in birds (Mengden, 1981). This linear pattern does not,
however, show a simple inverse relationship to the G-band pattern, though a comparable linear differentiation of the W is apparent when specific fluorochromes, such as DAPI and chromomycin, are applied in competition with counterstains. Such fluorochromes are thought by some workers (Schweizer 1980, 1981 for review) to characterize DNA with a predominant base pair composition. If this proves genuinely to be the case, it may eventually explain the linear differentiation of C-band characteristics. Certainly preliminary experiments on the in situ hybridization of the BK minor satellite DNA of the elapid Bungarus fasciatus to the W of Pseudechis porphyriacus (see Fig. 4.2) suggest a correlation between areas labelled by this satellite and the darker C-positive bands.

The patterns of lighter and darker C positive bands appear to be taxon specific (see Fig. 4.1). However, because of the complexity of the patterns and the inability to determine how differences in pattern relate to one another, it is not practicable at this time to utilize sex chromosome morphology and infrastructure in constructing phylogenies. Although the presence of a novel W chromosome morphology may suggest to the investigator that he is dealing with a distinct taxon, the case of the highly polymorphic Cryptophis nigrescens and intraspecific variation in Furina diadema both serve to caution against the de facto use of W chromosome morphology as a specific character.
(2) **Z-chromosome differentiation**

The variation of the Z chromosome of elapid snakes is most apparent from the banding data obtained for a variety of species. From the G-band data presented in Figure 6.4, we see that, whereas the five largest autosomes remain essentially unaltered in the species of *Drysdalia, Notechis* and *Tropidechis* illustrated, modification of the Z results in a reduction of its size in *Drysdalia* as compared to *Notechis* and *Tropidechis*. The latter two genera also show an alteration of centromeric location on the Z when compared to one another. The Z chromosome is also obviously highly modified between non-Australian elapid species (compare *Naja* Fig. 3.1, *Aspidelaps* Fig. 3.6 and *Ophiophagus* Fig. 3.9). In the Australian elapid *Suta suta* males have been found to exhibit heteromorphism between the two Z chromosomes within each individual with respect to the size of a block of telomeric heterochromatin. This results both in a difference in centromere location and in overall length between homologues when viewed in gross karyotypic preparations. Preliminary data, though from admittedly few specimens, suggests that females may inherit only the small, more submetacentric type of Z since the specimens examined all carried a Z of this type.
6.2.2. Microchromosome Evolution

Since their discovery microchromosomes have been treated by some authors as a distinct class of chromosomes in a manner similar to B-chromosomes. Newcomer (1963) has even doubted the chromosome nature of the micro-elements regarding them as acentric, variably heterochromatic and prone to fusion and fragmentation. They are on the contrary most certainly centric, they sometimes vary in heterochromatic content within a species and frequently do so between species and, as we shall shortly see, are subject to the same categories of chromosomal rearrangements as are also found in the macrochromosomes.

Their only truly distinctive character, therefore, appears to be their extremely small size.

Their appearance in some fish, many but not all bird and reptilian species and in all monotreme mammals, has confounded the efforts of some workers to derive a possible ancestry for such chromosomes. Ohno (1970) has expressed the opinion that these elements are of common ancestry and represent a line of karyotypic evolution distinct in some ways from other vertebrates. In fact, however, no hard data has been presented to demonstrate or refute homology in structure or function between the microchromosomes observed in the various vertebrates so that there is no evidence that these elements are conserved between major vertebrate groups.

Some early workers appeared to regard the reported differences in numbers of microchromosomes, both with and between related species, as indicative of their expendability. However, there is no good evidence that they do show intraspecific variation in number unless they have been
involved in structural rearrangement and it is clear that they are subject to the same types of rearrangements as all other elements in the genome. This latter conclusion is particularly defensible in light of the data on chromosome variation in elapid snakes presented here. For example, in the genus *Pseudonaja* related species can be shown to differ chromosomally only in the number and banding character of the microchromosomes. Such differences can be shown to be due to Robertsonian rearrangements as well as to heterochromatic additions (see *Pseudonaja*, Chapter 3). A case can be argued for multiple rearrangements of a similar character affecting only this "class" of chromosome because in some species most of the microchromosomes appear to have undergone the same type of change while the macrochromosomes exhibit an unchanged banding pattern (see C-block differences and Robertsonian changes in the *P. nuchalis* species group and *P. affinis*, Chapter 3).

The significance of the data on microchromosomes in reptiles is best understood in the light of the literature on avian systems from which our "knowledge" of the function and behaviour of microchromosomes is almost wholly derived. Slizynski (1964), for example, has suggested three ways in which microchromosomes are expected to behave differently from macrochromosomes:

1. **Movement.** Microchromosomes should move more quickly and more easily than macros during pairing and segregation.

2. **Chiasmata.** Microchromosomes are expected to have either one chiasma or none at all. Here Slizynski suggests that the
absence of chiasmata "would not lead to numerical inconstancy since the chromosomes are held together by stainable substance accumulated at their ends". This simplistic view suggests that micro-elements are in some way homogeneous and may refer to the clumping behaviour observed by early workers, resulting from poor fixation (Ohno et al., 1962) or NOR associations (Nashida et al., 1980).

(3) **Structural alterations.** Microchromosomes are expected to have predominantly single chromosome breaks which would make intrachromosomal structural changes rare.

While these assumptions were both logical and consistent with the limited data at the time they were proposed, little opportunity has been taken to test them. Several workers had already reported an apparent dichotomy between the behavioural patterns of macro and micro-elements. Schmid (1962) and Donnelly and Newcomer (1963), for example, had demonstrated that the microchromosomes of the chicken took up tritiated thymidine in early S phase. Galton and Bredbury (1966) subsequently showed the microchromosomes of the pigeon to be late replicating. On the other hand, pulse labelling through S phase in chicken bone marrow cultures showed no difference in DNA synthesis time (Bianchi and Molina, 1967). In the quail, Commings and Mottoccia (1970) showed that some, but not all, of the microchromosomes which possessed heterochromatin were GC-rich and late replicating and associated with the nucleolus. Brown and Jones (1972) explained the apparent restriction of a GC-rich repetitive DNA fraction to the microchromosomes of quail, as demonstrated by *in situ* hybridization, to be
consistent with the localization of this satellite in heterochromosomes and suggested the specific association of such a satellite with the nucleolar organizers. C-banding data of Stephos and Arrighi (1971) and Stock et al. (1974) indicates that the apparent discrepancies in replication patterns in the chicken, quail and dove could easily be explained by differences in heterochromatic content among the microchromosomes.

As this thesis shows, radical differences in the heterochromatic content of the microchromosomes is clearly present in related species of Pseudonaja. Heterochromatic addition to micro-elements has also been suggested as a contributing factor to karyotype change in the New World Natricine snakes which, by contrast to most elapids, exhibit few or no microchromosomes (Mengden and Stock, 1980).

The presence of nucleolus organizer regions (NORs) among the microchromosomes of birds was first demonstrated in the chicken after staining with Feulgen-light green (Ohno et al., 1962). Here some 12 microchromosomes were thought to be involved in nucleolar formation. The work of Commings and Mattoccia (1970) and Brown and Jones (1972) demonstrated an association between GC-rich heterochromatic regions and NOR's in the micro-elements. In situ hybridization of H\(^3\) labelled rRNA showed microchromosomes in the quail to possess ribosomal cistrons (Knibiehler et al., 1977). Using N-banding techniques 10 micros were assumed to carry NOR's in both the quail and the flamingo (Funaki et al., 1975) while in the pigeon most micros were both C- and N-band positive (Raman et al., 1978). More recently silver staining of five species in three genera of cranes demonstrated silver positive sites...
consistently on a single pair of micro-elements and these are often seen to associate in mitotic preparations (Nashida, 1980). The presence of a secondary constriction on the largest microchromosome in the varabilis species complex of Sceloporus lizards (Cole, 1978) suggest the presence of a NOR on the microchromosomes of some lizard species. From the data presented here on elapid snakes (Fig. 6.1, 6.2, 6.3 and see NOR discussion below) we see that the African and Asian, but not Australian elapid snakes, possess NOR's on one pair of microchromosomes as demonstrated both by silver staining and in situ hybridization of an 18 + 28S rRNA probe. This pair also shows association in a high percentage of mitotic cells. Thus in a number of birds, some lizards and in some but not all elapid snakes, one or more of the microchromosomes can be shown to possess NOR's.

Matthey (1975) proposed a theory to account for the derivation of the karyotype of those birds, reptiles and mammals possessing distinct macro and micro-elements from a progenitor karyotype which exhibited a gradual decrease in size of the chromosomes but with no clear distinction between micro and macro categories. This theory invokes multiple fusions which transfer median sized elements onto the larger chromosomes, thus creating a large size gap between the enlarged chromosomes so produced and the unchanged smaller elements. In a review of the available gross karyotypic data for birds Tegelström and Ryttman (1981) have concluded, without reference to Matthey's (1975) hypothesis, that the smallest macrochromosomes are created by Robertsonian fusions of larger micros and that the translocation of micros to existing macros
Figure 6.1  Ag-staining for nucleolar organizer regions in African and Asian Elapids.  (A) - *Hemachatus haemachatus*, showing association of the two Ag positive microchromosomes presumed to carry the NOR.  (B) - *Hemachatus haemachatus*, cell showing Ag positive microchromosomes not associated.  (C) - *Naja haje*, arrows indicate Ag positive sight on a pair of microchromosomes.  (D), (E), (F) - *Ophiophagus hannah*, showing Ag positive microchromosomes in both associated and non-associated states.  See text section 6.3.2 and species descriptions Chapter 3.
Figure 6.2. **In situ** hybridization of a 18s + 28s rRNA probe to the chromosomes of some elapid snake species. (A) - The frog *Litoria infrafrenata* used as control. (B) and insert (B') - *Acanthophis antarcticus*. Note labelling at the secondary constriction on one arm of both homologues of pair 1. (C) - *Hemachatus haemachatus*, note probe hybridizes to a pair of microelements and compare this to the Ag-staining in Figs. 6.1 A and B for this species. (D) - *Acanthophis* Adelaide River specimen, note probe hybridizes to only one homologue of pair 1. This specimen is heteromorphic for the secondary constriction on pairs 1 and 2 and homomorphic for a constriction on pair 6. See summary illustration Fig. 5.5, Chapter 5. (E) - *Cryptaphis nigrescens*, note hybridization of probe only to the secondary constriction on one arm of both homologues of pair 1 despite an N-band positive reaction in the heterochromatic regions of three pairs of acrocentric autosomes. (F) - *Tropidechis carinatus*, again the probe labels only the secondary constriction sight on pair 1.

One pattern that emerges is the localization of 18 + 28s rRNA probe to a pair of micro elements in African and Asian species, while in Australian species hybridization is restricted to the secondary constriction on pair 1. Only the Australian genus *Demansia* does not conform to this pattern and this genus has been demonstrated to be unlike all other forms inhabiting that continent.
Figure 6.3  N-band data for some Australian elapid snakes (A) Hemiaspis signata, (B) Pseudechis guttata, (C) Cryptophis nigrescens, (D) Denisonia devisii, (E) Suta suta. In each instance both the centric C-bands and those differentiating the W chromosome appear N-band positive. Additionally the secondary constriction on pair one reacts positively. These data compared with the in situ hybridization data illustrated in Figure 6.2, suggest that in snakes the N-band technique is not specific for nucleolar organizer regions.
results in the observed change in centromere position within
the macros from a telocentric to a submeta- or metacentric
position. To support this hypothesis the authors cite the G-
band work of Stock et al. (1974) which indicates that the
difference in G-band patterns between the pigeon and dove is
the result of a fusion involving four pairs of micros of the
pigeon which thus give rise to two small metacentric macro
pairs in the dove.

Although this translocation model appears to be the sole
mechanism proposed for microchromosome change in birds, Mengden
and Stock (1980) have provided clear evidence to suggest that
the secondary inter-conversion of macro and microchromosome
categories has also occurred by changes in heterochromatin
content in both boid (Sanzinia) and colubrid (Thamnophis)
genera. The banding data on elapid species presented in this
thesis demonstrates that acrocentric micro-elements certainly
appear to undergo centric fusions to give rise to metacentric
micro-elements (Pseudonaja). Additionally translocations of
micros to macro-elements have evidently also occurred which
lead to an increase in the overall size of the macros
(Hoplocephalus bungaroides and H. bitorquatus). Even so,
there is also evidence that the secondary inter-conversion
between micro and macro-categories may result from alterations
in heterochromatic content. Evidence from C- and G-band data
in Cryptophis nigrescens (Chapter 5, Section 5.2) confirms that
such heterochromatic addition may even occur as a within-
species event giving rise to a heteromorphic macro-element in
several specimens (Figs. 5.9 and 5.10). A similar addition,
resulting in a heteromorphic macrochromosome pair can be seen
in the same species (Figs. 5.11 and 5.14). Thus we see that a broader concept of macro and micro-chromosome formation must be applied to serpents than has previously been suggested for birds. In the light of these observations we can reach five general conclusions regarding microchromosomes:

(1) Size, and size alone, appears to be the only consistent character shared between the micro-elements of birds, reptiles and monotremes.

(2) Some micro-elements in most birds, some lizards and the African and Asian elapid snakes have been shown to possess NOR's and for this reason these share staining patterns associated with the NOR but this is not a universal property of micros.

(3) Differences in heterochromatic content may explain earlier interpretations ascribing distinctive replication patterns to the micro-elements in birds. While some elapid snake species show characteristic DAPI negative fluorescence in the micro-elements (see Chapter 4 and Fig. 5.6) this is consistent with their heterochromatic content.

(4) In some instances, such as varanid lizards (King and King, 1975) Sceloporus (Cole, 1978) and Pseudonaja species (present study), the micro-elements appear to be undergoing reorganization either independently to, or in a different direction from, the macro-elements. In the present study banding details have demonstrated the stability of the macrochromosomes
and the plasticity of the micros in some instances, e.g. Pseudonaja.

(5) Variations on the multiple fusion/translocation model provide the only explanation offered to date for the interconversion of the macro and microchromosomes classes (Matthey, 1975; Tegelström and Ryttman, 1981). While fusions and translocations are clearly operative within and between both of these size categories in elapids the data from this thesis, coupled with the work of Mengden and Stock (1980), demonstrate unequivocally that alteration of heterochromatic content is also responsible for the secondary interconvertability of these two chromosome size classes in at least some snakes.

What then do these conclusions suggest regarding the function of microchromosomes? If the centromere serves to inhibit crossing over of genes closest to the centromere, as it is known to in Drosophila, then the presence of a large number of these small linkage groups, consisting as they do of little more than a centromere and a small portion of chromatin, which is sometimes heterochromatic, would ensure that any genes located on a micro-element would have a higher probability of being inherited as a tight linkage unit. In such a situation a metacentric microchromosome would presumably be even more effective since both arms would be affected in the same way. The bimodal character of the genome could thus provide both for variability (macrochromosomes) and conservation (microchromosome elements). The addition of heterochromatin to the microchromosome would be expected to enhance gene integrity since heterochromatin is known to inhibit crossing over in its
immediate vicinity. This proposed function for a large number of small linkage groups within the genome is consistent with present day knowledge of the effects of centromere position and heterochromatin on crossing over. If the behavioural characteristics of microchromosomes proposed by Slizynski (1964) (see above) prove correct these would simply enhance this theory.

6.2.3 Heterochromatin Variation in the Autosomal Macrochromosomes

Unlike the heterochromatic changes which have occurred between species in both the sex chromosomes and the microchromosomes, the autosomal macro-elements appear surprisingly stable in heterochromatic characteristics. Certainly, there are subtle differences in C-band character between species within a genus, but instances of large scale heterochromatic addition of the type seen in *Cryptophis nigrescens* are indeed rare and there are few instances of interstitial C-bands other than those associated with the NORs. Interspecific variation is usually restricted to changes in amount and location of small telomeric blocks of heterochromatin as in the *Unechis gouldii* species complex (see Chapter 3) or the presence of procentric nodules. One notable exception to this generalisation is the genus *Acanthophis*. Here however prominent C-band differences are associated with the secondary constrictions in pairs 2 and 6. These secondary constrictions need not indicate NOR's since they do not label following in situ hybridization of the 18 + 28S rRNA probe and exhibit varying response to banding and fluorescence techniques. The C-band
positive material associated with these secondary constrictions appears to be G-band positive in the case of *A. pyrrhus*. They also vary in response to DAPI treatment. Clearly the material responsible for the C-band reaction is not of a homogeneous nature.

Another interesting variant in heterochromatin content is the appearance in several unrelated genera of elapids of large and very distinct centromeric blocks of heterochromatin in specific autosomes. These blocks are apparent for example, in pairs 2 and 3 of *Walterinnesia* (Fig. 3.7) and *Ophiophagus* (Fig. 3.9) and in all the autosomal elements of *Hemachatus* (Fig. 3.4) and the sea snakes *Laticauda colubrina* (Fig. 3.48) and *Disteria major* (Fig. 3.47). In *Hemachatus* and *Ophiophagus*, where both G-band and fluorescence data are available, these blocks react positively to both treatments. Whether this implies that these blocks represent a shared centromere specific satellite DNA is not known.

6.3 Nucleolar Organizer Regions in the Elapid Genome

The wide variety of species examined in this thesis, coupled with the application of almost every available technique to identify and characterize the NOR's, provides an opportunity not only to assess the interspecific variation but in some cases also to compare the relative value of the techniques employed.
6.3.1 The Techniques

Three techniques are widely used for localising nucleolar organizers in mitotic cells. These are N-banding (Matsui and Sasaki, 1973; Funaki et al., 1975), silver staining (Goodpasture and Bloom, 1975; Howell et al., 1975 and Bloom and Goodpasture, 1976) and localization of ribosomal genes by in situ hybridization with an 18S + 28S rRNA probe (see Henderson et al., 1972 and Evans et al., 1974). To assess their accuracy, the two former techniques were tested against the latter in a variety of species (Funaki et al., 1975 for N-banding and Goodpasture and Bloom, 1975 compared to Hsu et al., 1975 for silver staining). None of the published studies have, however, employed all three techniques (but see White et al., 1982 in press and this thesis).

The correspondence between N-banding and NOR location in some mammalian chromosomes (Matsui and Sasaki, 1973) has been interpreted as a reaction to specific residual proteins linked to the NOR. This interpretation rests on the assumption that the technique of N-banding involves the extraction of histone proteins (Matsui 1974a-c) and, as such, can be expected to stain a structural element rather than a gene product (Matsui, 1974b). Faust and Vogel (1974) have argued against such an interpretation, suggesting that N-bands do not correspond directly to the NOR but rather to the heterochromatin associated with the NOR. In an attempt to refute this criticism Funaki et al. (1975) undertook a survey comparing N-banding results with published in situ data. Of the 21 animal species analysed, 4 showed N-banding of the centromeres only. Some of the other species showed N-bands of the
telomere and "satellite" regions. These were also C-band positive in those cases that I have been able to check in the literature. N-bands were associated with secondary constrictions in only 12 species. These areas incidentally are also often heterochromatic. Funaki et al. 1975 interpreted these findings as concordant with their original assumption though Faust and Vogel (1974), on the other hand, have pointed out that (1) the human chromosome 14 possesses an additional N-band that is not known to be an NOR, (2) that N-bands were not detected in Microtus oeconomus and Eliomys quercicus even though they are expected to have an NOR, and (3) in M. oeconomus the chromosomes even show "satellite" associations in mitosis. Funaki et al. (1975) have provided a variety of explanations for these apparent discrepancies, including the quite remarkable statement that "the failure to detect an N-band does not necessarily imply its real absence".

The correlation with silver staining at first appears more defensible. Goodpasture and Bloom (1975), for example, reported a direct correlation between their silver data and the in situ data of Hsu et al. (1975), while Tantravahi et al. (1976) found an equivalent correlation in the higher apes. It has been suggested that the silver method stains a protein rather than DNA per se (Hubbell, 1979; Hubbell et al., 1979). Miller et al. (1976a and b) on the other hand, argue that silver staining depends not only on the presence of rDNA but also on the activity of the NOR itself. Thus, in mouse-human hybrids, where there is a repression of the human rRNA cistrons, the human acrocentrics known to possess silver positive NORs in conventional preparations fail to take up
silver in hybrid cell lines as they are not producing human 28s rRNA (Eliceiri and Green, 1969). There is also clear evidence for an increase in silver uptake paralleling an increase in nucleolar activity (Arrighi et al., 1980). Extensive data from human pedigrees demonstrates that silver stainability of the NOR is a heritable characteristic in maximally activated cells (e.g. cultured lymphocytes) and reflects both individual differences in the amount of rDNA and the capacity for NOR activation (Mikelsaar et al., 1977, Markovic et al., 1978).

Despite this close correlation between silver positive sites following Ag-staining and sites of in situ hybridization with 18 + 28S rRNA there are some apparent exceptions that warrant caution. In the gorilla there is an absence of both silver staining and a failure of in situ hybridization of the 18 + 28S rRNA probe although obvious secondary constrictions are present and these associate at mitosis. Tantravahi et al. (1976) suggested that this could indicate either an absence of rRNA genes or their presence in too few copies to be detected by these techniques. The latter event, however, could be explained by assuming that there would be a lower limit of resolution with these techniques. Additionally, silver staining has been shown to react both with loops of the lamnprush chromosomes of Triturus which are not NOR's, and stained sites in the Xenopus laevis genome, not previously thought to be associated with an NOR (Varley and Morgan, 1978). In Triturus vulgaris Nardi et al. (1978) found that while all sites that labelled with the 18 + 28s probe also silver stained, other sites were silver stained as well. Hubbell and Hsu (1977) showed that in neoplastic tumor lines
where there is a radical increase in the number of chromosomes, including those which bear NOR's, the number that stain with silver (6-9) remain at control levels. Again these data indicate that "silver staining in humans may not identify all NOR's that contain structural ribosomal genes". White et al. (1982 in press) have examined the parthenogenetic grasshopper *Warramaba virgo* and its relatives using N-banding, silver staining and *in situ* hybridization. They report a close correlation between N-banding and the sites of hybridization of the 18 + 28s probe but find that silver does not stain these sites, though it does stain the 5s rDNA site in one group.

From the data presented in this thesis N-banding certainly does not identify NOR's in snakes. It is my impression that the technique of Gerlach (1977), which was used by White et al. (1982) and is very similar to that of Funaki et al. (1975), results in nothing more than a modified C-band reaction in vertebrates. This incidently would be consistent with observations presented by Faust and Vogel (1974). Thus the technique is essentially nothing more than Geimsa staining following a hot 96°C 1N NaH₂PO₄ treatment. The prolonged treatment of slides with 65°C 2 x SSC is a principal step in C-banding and this solution is very similar to the buffer used in N-banding. By comparing the N- and C-band data for *Cryptophis nigrescens* it is evident that there are prominent blocks on several pairs of acrocentrics, as well as the secondary constriction on pair 1, which all react positively to both treatments (Fig. 5.13, 5.10). Yet *in situ* hybridization of an 18 + 28s rRNA probe labels only at the secondary constriction on pair 1. N-banding also results in a linear differentiation
of the W that correlates directly to the darker C-blocks reported in Chapter 4 (Fig. 4.1). These sites, of course, do not hybridize with the 18 + 28s probe; nor do they silver stain. Indeed, the presence of an NOR on the W might be expected to cause dosage problems. Also, in Acanthophis, where a wide variety of secondary constrictions are evident between the species and where these sites and the sex chromosomes have been found to N-band, the 18 + 28s probe again binds only to the secondary constriction on pair one (Figs. 6.2 and 5.3). All these findings are consistent with the conclusion that the N-band treatment simply labels only the most resistant C-blocks and these may or may not be associated with an NOR. Obviously, many NOR's show associated heterochromatic blocks and the coincidence of these observations may be responsible for earlier suggestions that N-banding depicts NOR sites.

The silver staining data on the elapid snakes are completely consistent with the results of the 18 + 28s rRNA in situ hybridization experiments. That is, in Australian elapids only the secondary constriction on pair 1 stains with silver, whereas in the African and Asian elapids a pair of micro-elements both silver stain and label with the 18 + 28s rRNA probe (Figs. 6.1, 6.2).
6.3.2 Major Trends in the Variation of NOR's in Elapids

Secondary constrictions have been used as markers for assessing species groupings by herpetologists dealing particularly with taxa that show little variation in gross karyotype. For example, almost every cytotaxonomic study concerned with the chromosomes of anuran amphibian's uses this marker. A notable example is Bogart's (1972) phylogeny of the genus *Bufo* as expanded by Formas (1978). Though a few workers have cautioned against the assumed stability of this character it remains the major marker used in the cytotaxonomy of the Ranidae (Haertel *et al.*, 1974; Corcoran and Travis, 1980; Early, 1971; Green *et al.*, 1981). In terms of the data available for *Acanthophis* I have already cautioned against characterizing secondary constrictions by a single property, such as gross morphology, C-band reaction or silver staining. It is apparent from the data on *Acanthophis*, that some of the variation in secondary constrictions is unrelated to changes in nucleolar organizer regions. Likewise in the case of *Acanthophis* though differences appear to characterize individual taxa, the secondary constrictions involved do not always show homology with regard to their banding and fluorescence behaviour. I have already listed evidence indicating that, in humans at least, NOR expression is a heritable property. It has been proposed that changes in location of NOR's can occur either from the translocation of all or part of existing NOR's or by a process of amplification of presumably repressed pre-existing sites. Evidently the use of secondary constrictions as a taxonomic character in cases
where homologies cannot be demonstrated by banding or fluorescence (King, 1980; Schmid, 1978) must be regarded as highly speculative. With these facts in mind, it is worth reiterating the major trends in the variation observed in the secondary constrictions of elapid snakes:

(1) The Australian Terrestrial elapids appear highly conservative exhibiting a prominent secondary constriction on pair 1. This constriction has been identified as a genuine NOR following in situ hybridization with a 18 + 28s rRNA probe. Moreover this NOR is maintained and functional even after a centric fission event in chromosome 1 in *Hemiaspis signata*. Where other secondary constrictions occur, as in *Acanthophis*, these do not label with the rRNA probe.

(2) The only group of Australian terrestrial elapids which do not show this pattern are species of the genus *Demansia*. As has been pointed out earlier, *Demansia* differs radically from all other Australian terrestrial elapids in gross karyotype and in banding properties. Species of this genus possess no obvious secondary constriction. Attempts are currently underway to identify the location of the NOR in this group. This genus has also been found to differ from all other Australian elapids in the MC'F work of Cadle and Gorman (1981).
(3) All the hydrophid sea snakes reported here and elsewhere in the literature exhibit a secondary constriction on pair 1. There is to date, however, no G-band data available to suggest that this chromosome is conserved between the Australian elapids and the sea snakes. A secondary constriction is not obvious in either the gross or the C-band preparations presented here for the laticaudine sea snake *Laticauda colubrina*, nor was such a constriction reported by Gorman (1981) in describing the gross karyotypes of three species of this genus.

(4) The gross karyotypes of all new world coral snakes display a prominent secondary constriction on pair 1 (Gutierrez and Bolanos, 1979; Graham, 1977 and present study). However, even gross preparations indicate that both this chromosome and the rest of the karyotype have undergone considerable change compared to the Australian elapids (Fig. 6.9). Consequently it is impossible to make assumptions of broad homologies from data of this kind.

(5) African and Asian elapids for which in situ and banding details are known, possess an NOR on one pair of microchromosomes (Fig. 6.1 and 6.2).

In the light of these data, and because banding studies have not been carried out for all the groups mentioned, I am reluctant to attempt to assess the phylogenetic weight of these trends in NOR location. The pattern that emerges does,
however, suggest that the continentally endemic elapids appear highly conserved with regard to the location of the NOR though these continental groups differ from one another. The hydrophid sea snakes are more clearly associated with the Australian terrestrial elapids while the Asian elapids are more obviously related to the African forms.

6.4 Inversions, Translocations and Robertsonian Rearrangements

The data on elapid snakes does not provide evidence for any novel mechanisms as far as structural chromosome changes are concerned. That is to say, although these types of change are without doubt responsible for the majority of the variation observed between taxa there is nothing in the data that expands our knowledge of the mechanisms *per se*. I have reported on these changes in the descriptive Chapter 3, and will refer to them again in the following sections on species groupings and cytotaxonomy.

6.5 Criteria for Groupings Based on Chromosomal Data

As outlined in section 1.2.1 of Chapter 1, there are two distinct approaches to the use of chromosomal data when interpreting possible taxonomic relationships between organisms. Wherever possible the first step is to divide the specimens into karyomorphic groups based on shared fixed differences. The second and more tenuous step is to attempt to derive a phylogeny, or interpretation of the intergroup relationships,
based upon the degree of complexity of steps necessary to derive them from a presumed ancestral karyomorph (see section 1.2.1). Some of the trends evident from the descriptions in Chapter 3 clearly suggest potential karyomorph groups. Intrinsic to the definition of such related groups, however, is the ability to detect the degree of homology between karyotypes. While the banding results presented in this thesis provide a clear advantage over the simple gross karyotypic data used by all previous workers on snakes, it is appropriate here to outline the approach I intend to use for the determination of homology.

The review of Seuanez (1979) proposed the most comprehensive and parsimonious approach to defining chromosome homology based on a wide variety of banding details. Seuanez (1979) summarised the degrees of interspecific similarity between chromosomes in the following terms:

(1) between chromosomes with identical morphology and G-banding pattern.

(2) between chromosomes of very similar (but not identical) morphology, but showing practically identical G-banding pattern. Morphological change can then be due to (a) the amount or location of constitutive heterochromatin, (b) the amount of brilliant fluorescent material or (c) the amount of highly repetitive sequences.
(3) between chromosomes of different morphology and C-banding patterns which, however, can be derived from one another by chromosomal rearrangement.

(4) between chromosomes of similar morphology but with G-banding patterns not strictly coincident that cannot be derived from each other by chromosome rearrangement.

This assessment clearly highlights the importance given to G-banding data in human and mammalian cytogenetics in general. As has been stated earlier in this thesis, in most G-band studies, there is an underlying assumption, which has not been properly tested, that there is a direct correlation between G-band pattern and gene content. Regardless of our ability to demonstrate the precise relationship between gene content and G-banding the mass of G-banding data on human birth defects certainly attests to its value in detecting some types of chromosome structural rearrangements.

Throughout this thesis I have demonstrated that a variety of banding and fluorescence techniques suggest that heterochromatic regions, and the secondary constrictions often associated with them, contain a heterogeneous group of materials at least in relation to their staining properties. Thus, while the wide variety of techniques applied do allow a better characterization of interspecific differences in heterochromatin content they do not allow unequivocal demonstration of homology between specific blocks of heterochromatin. This, coupled with our knowledge of the individual variability of heterochromatin in defined pedigrees suggests that, though a
knowledge of the location and size of G-band regions is necessary for interpreting changes in gross karyotypic morphology, they may not always provide an accurate marker of homology. Though the location of heterochromatic regions appears to be relatively stable in most snake species, the fact that they are clearly subject to amplification in others suggests that they are maleable at least in some instances and thus warrants caution in their use as markers.

In the present discussion G-banding details are relied on heavily to determine homologies and the types of rearrangements that have occurred between groups. The groupings based on structural differences have been arrived at with a knowledge and consideration of the effects of heterochromatic differences on both the gross karyotype and its G-band character. The reader must treat with caution character groupings and relationships of species reported here for which I have not been able to obtain extensive banding data, as well as my attempts to incorporate the published data of other workers into these groupings.

6.6 Phylogeny of Elapid Snakes

6.6.1 Species Groupings in Australian Elapids Based on Chromosomal Similarity

The following species groupings are derived largely from the data presented in Chapter 3, though within and between group comparisons of the G-band data is presented in the
discussion here. As the bulk of the data available is from Australian species I will first characterize the groups to which they belong.

**Australian Elapid Species Groups.** Those genera for which G-banding data is available are designated by the suffix G:

**Group 1**

$2n = 36 = 16M/20m$, sex chrom. = pr. 4, three smallest macros are acrocentric

- *Pseudechis butleri* G
- *P. guttata* G
- *P. porphyriacus* G Fission pr. 3 *P. australis* (38, 18M/20m)

- *Acanthophis antarcticus* "Southern Form" G
- *A. antarcticus* Barkley Tablelands G
- *A. antarcticus* Adelaide River G
- *A. praelongus* G
- *A. pyrrhus* G

- *Echiopsis curta*
- *Rhinoplocephalus bicolor*
- *Vermicella annulata*
- *Neelaps bimaculata*
- *Drysdalia coronata*
- *Cacophis krefftii G* C. squamulosus G
While some genera, sharing the group 1 karyomorph, such as Acanthophis and Echiopsis on the one hand and Vermicella and Neelaps on the other, have been suggested in the literature to be related on morphological grounds, both are clearly highly specialized morphologically. Others, such as Rhinoplocephalus, are of unknown relationship. It appears therefore that, in overview, this karyomorph is largely shared by a variety of unrelated species. It is thus more reasonable to assume that they have conserved a primitive condition chromosomally while diverging morphologically than to suggest that they have converged to a common karyomorph. It is important, therefore, to recognize that the maintenance of a primitive character state need not denote relationship between those sharing it. A comparison of the G-banded chromosomes of the species of this group for which data are available appears in Fig. 6.7. Pseudechis australis (2n = 38 = 18M, 20m) is derived from its congenitors within this group by a fission of metacentric pair 3 to give rise to acrocentric pairs 8 and 9 as outlined in Chapter 3, section 3.5.6. The species Cacophis squamulosus, with 2n = 36 (16M, 20m), would then be derived from its congenitors within the group by pericentric inversions on the pairs 6, 7, and 8 giving rise to subacrocentrics in place of the acrocentrics typical of this karyomorph (see Section 3.5.16). The derivation of other related karyomorphs from this presumed ancestral condition are given below.
Group 2

2n = 36 = 16M/20m, sex chrom. = pr. 4

Oxyuranus scutellatus

O. (Parademansia) microlepidota

Though differing from one another in C-band details (see Sections 3.5.3 and Fig. 3.16) the karyotype of these two species is very similar and quite unlike the 2n = 36 forms of Pseudonaja. As such, the karyotype of O. (P) microlepidota differs from that of the Pseudechis in "Group 1" above in possessing a metacentric pair 8. O. scutellatus possesses a pair 8 with an apparently identical morphology but differs further in the presence of a subacrocentric pair 7 as opposed to the acrocentric pair 7 in both "Group 1" species and O. (P). microlepidota.

Group 3

2n = 38 = 18M/20m, sex chrom = pr. 4, 5 smallest macros are acrocentric.

Simo selaps bertholdi  G
S. warro

These two species are essentially identical in gross karyotype. Banding details are available only for S. bertholdi (Fig. 4.13.30). The karyotype of these two species can be derived from the supposed ancestral condition,
maintained in Neelaps, by a centric fission of metacentric pair 5. It is interesting to note that the two Simoselaps species possess minute short arms on three of the small acrocentric pairs as too does Neelaps (compare Fig. 3.30 and 3.32). This is not seen in other members of "group 1" but the paucity of banding data for the species in question prevents me from expanding on these observations.

Group 4

2n = 34 = 14M/20m, sex chrom. = pr. 4, smallest macro pair is acrocentric.

Notechis scutatus G

N. ater ater G

N. a. niger G

N. a humphreysi G

N. a serventyi G

N. a. occidentalis G

Austrelaps superba (and races) G

Tropidechis carinatus G

While Groups 2 and 3 are easily derived from group 1, Group 4 may represent the core of a distinct chromosomal lineage interrelated to groups 5 and 6. G-band data summarised in Fig. 6.4 demonstrates a paracentric inversion difference in pair 7 between Notechis and Tropidechis and, with respect to this chromosome, Tropidechis is similar to Drysalia coronoides in "Group 5". The various genera in this group incidentally display only minor differences in G-band patterns.
Group 5

2n = 34 = 14M/20m, sex chrom. = pr. 4, smallest macro pair is acrocentric

Drysdalia coronoides G
D. mastersi G
D. rhodogaster G
Denisonia devisii G

D. maculata

As seen in Figs. 3.15 and 3.20, members of this group are chromosomally very similar. Moreover G-band comparisons demonstrate the chromosomes of "Group 5" species to be homologous in most instances to those of "Group 4" (Fig. 6.4). A modification to the Z chromosome (see Section 3.5.2) results in a size difference from the Z of the "Group 4" species. Thus autosomal pair 4 of "Group 5" is equivalent to pair 5 in the "Group 4" species. Denisonia devisii and D. maculata also appear chromosomally similar to the Drysdalia species in this group, though G-band data is not available for D. maculata. The two species of Denisonia could also, therefore be derived from the "Group 4" karyomorph by a modification of the Z.
Note that autosomal pair 4 of Drysdalia (Group 5) is equivalent to autosomal pair 5 of Notechis and Tropidechis (Group 4). Thus the difference in size of the sex chromosome pair is due to modification of the Z and not to an independent origin of the sex pair. See text section 6.6.1 for further discussion.
Group 6

2n = 30 = 16M/14 "m", sex chrom. = pr. 4, 3 smallest macro pairs are biarmed.

Suta suta  

Denisonia punctata  

Unechis gouldii  

U. monachus  

U. nigriceps (= brevicaudus)  

U. dwyeri  

The species in this group share a very distinctive karyotype and are similar to one another in G-banding pattern. Suta and some species of Unechis also share a unique heteromorphism in the C-band character of the male Z chromosome.

Group 7

2n = 36 = 20M/16m but poorly demarcated, 2n = 40 20M/20m polymorphic.

Unechis carpentariae  

Cryptophis pallidiceps  

C. nigrescens  

U. nigrostriatus  

The karyotypes of U. carpentariae and C. pallidiceps are unique among the 2n = 36 elapids and essentially identical to one another. Both possess six pairs of acrocentrics decreasing
gradually in size from pair 5 to pair 10 followed by a small metacentric pair resulting in the division between macro and micro categories being ill defined. The derivation of C. nigrescens from C. pallidiceps which results from a centric fission of pair 3 has already been discussed in section 3.5.7. and is confirmed by G-band comparisons between U. carpentariae and C. nigrescens (Fig. 6.6). The subsequent differentiation between macro and micro categories is a complex phenomenon in C. nigrescens and has been discussed in detail in Chapter 5.

The inclusion of U. nigrostriatus in this group must be considered tentative as there is no banding data currently available for this species. As outlined in section 3.5.11, the gross karyotype of U. nigrostriatus (2n = 34) differs from U. carpentariae in possessing one pair of metacentric micro-elements in place of two acrocentric pairs. U. nigrostriatus shows a similar decrease in size of the median elements, though these are predominantly biarmed. Without banding data it is impossible to determine if this difference is caused by heterochromatic addition or by inversion.

Group 8

2n = 42 = 22M/20m, sex chrom. = pr. 4, all macros biarmed.

Demansia atra

D. olivacea  G
D. papuensis
D. torquata
D. psammophis  G
Figure 6.5. G-band comparison of some Australian elapids possessing a diploid number of 30. Ud = Unechis dwyeri, S.s. = Suta suta, Pn = Pseudonaja nuchalis black-headed morph. Unechis and Suta compare well while Pseudonaja does not. Those chromosomes that are largely non-homologous between Pseudonaja and the other two genera are indicated by double lines.
Figure 6.6. G-band comparisons between some group 7 Australian elapids. Cp = *Cryptophis pallidiceps*, Cc = *C. nigrescens*, Uc = *Unechis carpentariae*, Ud = *U. dwyeri*. Double lines to the right of the figure indicate a lack of homology between those chromosomes of *U. dwyeri* and the other *Unechis* and *Cryptophis* species illustrated. The large amount of heterochromatin on some acrocentric macrochromosomes in *C. nigrescens* may make it more difficult to determine homologies. The illustration for *C. pallidiceps* presented here is of course a gross karyotypic preparation.
Figure 6.7. G-band comparison of some group 1 Australian elapids all of which possess a diploid number of 36. Pp = *Pseudechis porphyriacus*, Pa = *Pseudechis australis* (note the fission event equivalent to pair 3), Pg = *Pseudechis guttata*, Adl = *Acanthophis antarcticus* Adelaide River, N.T. specimen, Apyr = *Acanthophis pyrrhus*, Aa = *Acanthophis antarcticus* southern form.
This group is completely unlike all other Australian elapids in diploid number and mitotic configuration. C-band data demonstrates that the large number of biarmed macro-elements are not the result of heterochromatic addition.

**Group 9**

<table>
<thead>
<tr>
<th>Species</th>
<th>2n =</th>
<th>M/M =</th>
<th>Chromosome Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudonaja textilis</td>
<td>38</td>
<td>18/20</td>
<td>G</td>
</tr>
<tr>
<td>P. guttata</td>
<td>36</td>
<td>16/20</td>
<td>G</td>
</tr>
<tr>
<td>P. modesta</td>
<td>36</td>
<td>16/20</td>
<td></td>
</tr>
<tr>
<td>P. ingrami</td>
<td>36</td>
<td>20/16</td>
<td>G</td>
</tr>
<tr>
<td>P. affinis</td>
<td>34</td>
<td>16/18</td>
<td>G</td>
</tr>
<tr>
<td>P. nuchalis</td>
<td>34</td>
<td>16/18</td>
<td></td>
</tr>
<tr>
<td>P. &quot;nuchalis&quot; Black Headed Morph</td>
<td>32</td>
<td></td>
<td>G</td>
</tr>
<tr>
<td>P. &quot;nuchalis&quot; &quot;Darwin Type&quot;</td>
<td>30 = 16/14</td>
<td></td>
<td>G</td>
</tr>
</tbody>
</table>

The genus *Pseudonaja* shows the greatest degree of species-specific chromosomal variation in any elapid genus. Though banding data is available here for a majority of recognized species of *Pseudonaja* it is apparent that the mechanisms responsible for the observed variation are obviously complex. I must reserve the definition of intrageneric relationships for a broader survey of the genus which is currently underway. Suffice it to say that those *Pseudonaja* species sharing diploid numbers with the various karyomorph groups defined above do not resemble those groups in either gross morphology or banding details.
Group 10

2n = 28 = 14M/14m except in Z1, Z2, W females.

**Hoplocephalus bungaroides**

- *H. bitorquatus*  
- *H. stevensi*

This specialized genus exhibits what I interpret as a derived karyomorph with extensive reduction in microelements. There is, however, greater similarity between the G-banded macrochromosomes of these species and the "Group 4" species than with the 2n = 30 "Group 6" species.

**Non allocated species**

There remain two genera which have not been categorized into any of the above ten karyomorph groups. These are *Hemiaspis* (2 species) and *Furina* (polytypic). *Hemiaspis damelii* (2n = 34, 16 M/14m) could be derived from a 2n = 36 karyomorph, with minute short arms on pairs 6 through 8, by a simple fusion of one pair of microelements. *Cacophis squamulosus* (G) shows similar short arm differences compared to its Group 1 congenitor *C. kreffii* (G). This is not to say that *H. damelii* and *Cacophis* are of common lineage but simply that they could be derived from a similar archetype. As no G-band data exists for *H. damelii* I cannot place it with confidence. *H. signata* (2n = 40 G) can be derived from a similar ancestral karyomorph as its congenitor by two centric fissions prior to the presumed microchromosome change in
Group 1
Pseudochis butleri
P. guttata
P. porphyriacus
C. australis

Group 2
Oxyuranus (P) microlepidoia
O. scutellaia

Group 3
Pseudonaja

Group 4
Notechis

Group 5
Drysdalia coronata

Group 6
Vermicella
Rhinoplocephalus
Heelaps

Group 7
U. carpentariae
C. pallidiceps
C. nigrescens

Group 8
Drysdalia denisonia
D. maculata
D. devisii

Group 9
Demansia

Group 10
Hoplocephalus

Acanthophis
Echiiopsis

Cacophis kreffti
Cacophis squamulosus

U. punctata
U. gouldii SPS complex
H. damelii (see Section 3.5.13 and G-band Fig. 6.7).

The remaining genus Furina has been demonstrated to be polytypic (Section 3.5.12). Due to the small sample available, the absence of G-band data and the lack of comparative data on Glyphodon, I will not attempt to categorize this genus at this time.

The groupings which emerge based on chromosomal data alone can be summarised as shown on the facing page. The between-group relationships indicated are those most obviously suggested by chromosomal change from a 2n = 36 ancestor. I reiterate that the species within Group 1 maintain a "primitive" condition chromosomally but do not necessarily resemble the archetype in morphology. As has been mentioned in Chapter 1, Section 1.2.2., Gorman (1981) has interpreted the similarity in gross karyotype between Notechis (Shine and Bull, 1977) and that of one of the three laticaudine sea snake species as possibly indicating that these forms share a "primitive" karyotype and that this karyotype may be the ancestral condition for the entire elapid radiation. I wish to emphasize to the reader that even should this assumption prove valid it would not alter the groupings listed though it would alter the direction of between-group changes. From a phylogenetic standpoint, however, the assumption of a "Group 4" karyotype as ancestral would necessitate invoking a "canalisation" type model (Bickham and Baker 1979) to explain the convergence on a Group 1 karyomorph.

In order to determine the possible taxonomic significance of the ten principal karyomorph groupings, it is necessary to compare them to the existing groupings based on morphological
and chemotaxonomic data. I should mention that I am not in a position to weigh the relative values of these characters and wish simply to point to correlations.

The relationship between the two *Oxyuranus* species (Group 2) is clear and is based on scalation, dentition, skull morphology, hemipene anatomy and karyotypes (Covacevich et al., 1981). The venom studies of Saint Girons and Detrait (1980) and Southerland (1979) point to close similarity between the species of *Oxyuranus* and their distinctiveness from *Pseudonaja* (Group 8). The venoms of both these genera incidentally also have very weak cross reactivity with that of other elapids (Saint Girons and Detrait, 1980) suggesting they may be derived "out groups". The two *Oxyuranus* species are the only Australian elapids which share the combined scalation character of a single anal plate and divided subcaudals.

The distinctiveness of the *Demansia* species (Group 9), both chromosomally and in regard to the MC'F work of Cadle and Gorman (1981), has been mentioned repeatedly throughout this thesis and need not be reiterated here.

The cohesive nature of the Group 4 species is generally defensible on chemotaxonomic data. *Notechis* and *Tropidechis* possess very similar venom components (Morrison, in press). Minton and da Costa (1975) observed no reaction between venom of *Notechis* and antisera of *Austrelaps*. Likewise Coulter et al. (1981) in testing the venoms of most large Australian terrestrial elapids to anti-Notexin (from *Notechis*) using enzyme immunoassay found *Austrelaps* to give a reaction most similar to *Notechis* itself.

Group 5 species are apparently quite close to those of
Group 4 and may be associated through *Drysdalia*. McDowell in fact could find no internal morphological feature to separate *Drysdalia coronata* and *Notechis*. If this reflects a true relationship then *D. coronata* could be at the center of the chromosomal divergence of Groups 4 and 5, thus:

![Diagram]

<table>
<thead>
<tr>
<th>D. coronoides</th>
<th>D. mastersi</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. coronata Group 1 2n = 36 Fusion to 34</td>
<td></td>
</tr>
<tr>
<td>D. rhodogaster Group 5</td>
<td></td>
</tr>
<tr>
<td>D. devisii</td>
<td></td>
</tr>
<tr>
<td>D. maculata</td>
<td></td>
</tr>
<tr>
<td>Notechis</td>
<td></td>
</tr>
<tr>
<td>Austrelaps Group 4</td>
<td></td>
</tr>
<tr>
<td>Tropidechis</td>
<td></td>
</tr>
</tbody>
</table>

It should be mentioned again that Storr (in press) has synonymised *Drysdalia, Austrelaps* and *Notechis*. He also includes *Elapognathus* for which I have no chromosomes data but which is morphologically similar to *Drysdalia* and *Echiopsis*, which I place in Group 1.

The separation between *D. devisii* and *D. maculata* in Group 5 on the one hand, and *D. punctata* with *Suta* and the "*Unechis gouldii* species group" (group 6) is in complete agreement with McDowell's (1967) anatomical data. It has also become conventional to treat the "*U. gouldii* species complex" along with *Suta* as distinct from other *Unechis* species (Coventry, 1971 and Storr, 1981). It can be seen from Section 1.1.2 that all
Group 4 species, except Austrelaps, and most Group 5 species share a similar morphology of the abductor externus superficialis muscle while all Group 6 species share a difference in this character. It may also be noted that with respect to this character, Group 6 animals are similar to Group 1 Pseudechis and Group 4 Austrelaps. The association of Pseudechis and Austrelaps has been suggested on other occasions (McDowell, 1970). The discrepancies that exist between the chromosomal groupings listed above and the venom gland musculature of McDowell (1967) are not great. The larger number of species groups derived from the chromosomal data however suggest a higher degree of resolution than has been possible through the use of morphological data.

I am reluctant to expand upon the position of Cacophis or Furina until data becomes available from Glyphodon. This may also shed light on the relationship of Group 7 species to the others.

6.6.2 A Chromosomal Comparison of African and Asian Elapids

The banding data available for African and Asian elapid species is not as extensive as that presented above for Australian elapids. Consequently the determination of interrelationships is even more difficult. G-band comparisons between representative Naja species and the two monotypic genera Hemachatus and Ophiophagus, illustrated in Fig. 6.8, suggest some possible chromosome arm homologies, but such instances are rare and represent only a small percentage of the genomes. Thus, pair 1 appears conserved in these three
Figure 6.8. A comparison between the possible homologies in G-band patterns of some Asian and African elapid snakes. In this figure: O.h. = *Ophiophagus hannah*
H.h. = *Hemachatus haemachatus*
N.n.k = *Naja naja kaouthia*
N.h. = *Naja haje*
refer to section 6.6.2 for further discussion.
genera, excluding the presence of the G-band positive centric material in *Hemachatus* and its absence in the other two genera. A portion of pair 2 also appears similar between *Ophiophagus* and *Hemachatus*, though again excluding centric differences and the presence of a G negative block at the telomere of the long arm in *Ophiophagus*. This chromosome varies in *Naja* species by one or more inversions, resulting in a sub telocentric chromosome in the Asian *N. n. kaouthia* and a telocentric chromosome in the African species. Thus, only the distal portion of the long arm of pair 2 can be said to suggest homology. Pair 3 displays only vague homology between *Ophiophagus* and the Asian *Naja*. Two small acrocentric chromosomes in *Hemachatus* can be combined to suggest homology with pair 4 of *Naja*. Aside from these possible homologies between segments of chromosomal arms, the combined gross karyotypic, banding and fluorescence data demonstrate the remainder of the genomes of African and Asian elapids to be massively modified. This applies to both within and between continental comparisons of genera with the exception, of course, of *Naja* (see Chapter 3). Moreover, except in specific cases, such as *Walterinnesia* where centric fission of pair 1 can be demonstrated, the differences in chromosome number and morphology between genera are such as to obscure intergeneric groupings and homologies. The degree of variation at the species level within genera is more subtle (see *Aspidelaps* and *Naja*, Chapter 3). It will be recalled from earlier discussion that these genera may share similarity in the location of the nucleolus organizer region on a microchromosome pair.

The pattern that emerges then is one in which the genera
of African elapids are distinctive chromosomally. This appears to be particularly true of *Dendroaspis* which, incidentally, is the only African genus to display a \( 2n = 36, 16M/20m \) karyotype. The end result is that, with the data available, it is currently impossible to draw meaningful conclusions on the lineage relationships at least between African genera. It is not without interest that, on the basis of MC'F work (Cadle, pers. commun.), "the African genera *Dendroaspis*, *Naja* and *Elapsoidea* and the Asian *Bungarus* all appear well differentiated from one another, and do not form distinct clades (thus, in a phylogenetic tree, they appear as distinct lineages with little structure at the base of the tree)."

### 6.6.3 The Micrurine Elapids

Gross karyotypic data has been published for six taxa of New World Coral Snakes of the genus *Micrurus*. Diploid number varies from 26 to 34 with considerable variation in macro and microchromosome numbers. In the discussion above, I have presented a figure for *Micrurus fulvius tenere*. It is interesting to note that all *Micrurus* species possess a prominent secondary constriction on the chromosome equivalent to pair 1. This is true even in *M. alleni*, where this chromosome appears to have undergone centric fission, and *M. bipartatus*, where chromosome rearrangement has resulted in the production of a pair larger than the conventional unaltered "pair 1". The *Micrurus* genome is variable in respect of autosomal centromere position but, with one exception, consists of eight pairs of distinctly larger macrochromosomes followed
Figure 6.9. *Micrurus fulvius tenere* 2n = 34 (16M/18m).

Note the gross karyotype of this North American elapid is unlike that of any of the old world elapids reported in this thesis. While it does possess a secondary constriction on pair 1, this autosome differs from other elapids in centromere position. Refer to the text sections 6.3.2 and 6.6.3.
either by metacentric median elements or by a larger number of micro-elements. The micrurine elapids are represented by over 50 species in the neotropical Americas. The small number of species karyotyped thus far, the absence of banding data and the lack of their availability for study in Australia, prohibits any further assessment of the possible group relationships of these forms. Clearly, they have undergone diverse chromosomal rearrangements and would be an interesting group to study in terms of microchromosome evolution. I can, however, state with confidence that the karyotypic appearance of all the reported species of *Micrurus* bears no similarity to any of the other elapid species discussed here.

### 6.6.4 The Sea Snakes

The taxonomic relationships of the sea snakes have been discussed in detail in Chapter 1. Most workers consider the laticaudine sea snakes as distinctive from the hydrophines though they attribute varying degrees of taxonomic distinction at the familial or subfamilial level. The hydrophines are generally subdivided into a primitive *Ephalophis* type group, an intermediate *Aipysurus* group and the more derived hydrophiids. Both major groups of sea snakes are thought to be derived from terrestrial elapids. McDowell associates *Laticauda* with *Micrurus* and the old world coral snakes while the hydrophiids he affiliates with the "Demansia" group of Australian elapids, specifically *Drepanodontis* (= *Hemiaspis*). As has been commented on earlier, the genera McDowell has allocated to his "Demansia group" do not appear in any way related chromosomally.
Gorman (1981) has reviewed the gross karyotypic data for the 11 species of sea snakes for which chromosomal studies were previously available. These species all fall into either the genus *Laticauda* or the derived hydrophiids. In Chapter 3 I have described gross karyotypic and C-band data for six sea snake species - one *Laticauda*, one hydrophiid, *Disteria major*, and four species from the "Aipysurus group" - for which no chromosome data is currently available. As no G-band information is available for any sea snake species I am reluctant to discuss their inter and intrafamilial relationships. However, as none of the previous workers have displayed such reluctance in assessing relationships based on gross karyotypic data alone, I will attempt to show where the gross karyotypic and C-band data may fit into the previously reported literature.

Gorman (1981) demonstrated that the macrochromosomes of the two *Laticauda* species may be derived by Robertsonian rearrangement. Prior to such an event, the ancestral species would presumably have possessed a $2n = 34 = 14M/20m$ karyotype, with the smallest macro being acrocentric. As this is also seen in the published gross karyotype of the Australian terrestrial elapid *Notechis* (Group 4 see above), he suggested this may represent the "primitive" karyotype of the elapid radiation. It can be noted from the data presented here on *Laticauda colubrina* that although the gross karyotype is similar to *Notechis* it differs in possessing large centric C-blocks on all the macrochromosomes. Additionally no obvious secondary constrictions are present on pair 1 of *Laticauda*. Though the figure provided by Gorman (1981) may indicate a secondary constriction in one of the three *Laticauda* species
examined by him (*L. colubrina*) he made no comment on it.

Many of the hydrophiids reported previously possess a diploid number of 32 in the males though four show multiple sex systems in the females. *Disteria major* described here (Chapter 3) also possess 2n = 32 = 14M/18m with a ZW sex chromosome system similar to that seen in some, but not all, *Hydrophis* species as well as in *Kerilia*. There is a prominent secondary constriction on pair 1, but also a smaller constriction on pair 2. Both are C-band positive (Fig. 3.13).

Within the presumably intermediate "*Aipysurus* group" I have described gross karyotypic data from *Emydocephalus annulatus*, *Aipysurus duboisii*, *A. fuscus* and *A. laevis*. All possess a diploid number of 32 with 14M/18m and a ZW (pair 4) system. There is, however, no small acrocentric macrochromosome and the three smallest vary considerably. It is interesting to note therefore, that the "intermediate" "*Aipysurus* group" do not resemble the *Notechis* Group 4 karyomorph, while *Laticauda* and hydrophiids do, except however with regard to one pair of microchromosomes. Without further banding data from a variety of species it is ludicrous to hypothesise on whether this preliminary data suggest that *Aipysurus* occupies a tangential rather than intermediate position in hydrophid evolution.

In conclusion, it is apparent that, when banding data is available for the majority of species in a large family, these data can be used to advantage in deriving species groupings. A correlation between such species groupings and the chemotaxonomic and morphological data suggest they may indeed be taxonomically useful. When data from the remaining elapid
species become available it may well prove possible to construct a phylogeny that reflects both taxonomic and phylogenetic significance.

6.7 General Conclusions

In the taxonomic overview presented in the first chapter of this thesis, I raised questions at the familial (Section 1.1.1.) and generic (Section 1.1.2) levels concerning the relationships of the Australian elapids both to one another and to the family as a whole. I am now in a position to answer these questions and offer the following conclusions:

Section 1.1.1. Familial relations

(1) While the continentally endemic groups of terrestrial elapids appear for the most part chromosomally distinctive they nevertheless can be considered confamilial.

(2) The Australian elapids are clearly chromosomally distinct from the Asian and African elapids but whether this reflects familial recognition is doubtful since the Australian elapids are no more distinctive from their Asian and African counterparts than are the Micrurines of the New World.

(3) The Australian elapids are probably not monophyletic because of the distinctive nature of the genus Demansia.
(4) Some of the laticaudine sea snakes appear chromosomally similar to some of the hydrophids but their relationship to Australian terrestrial elapids is still not clear.

(5) There is no evidence to demonstrate unequivocally that the hydrophid and laticaudine sea snakes are of independent origin.
REFERENCES


Hubbell, H.R.: Cytological and Biochemical Aspects of Silver Staining. Dissertation, The University of Texas Health Science Center, Graduate School of Biomedical Sciences at Houston. (1979).


White, M.J.D., Dennis, E.S., Honeycutt, R.L., Contreras, N., Peacock, W.J.: Cytogenetics of the Parthogene-
tic Grasshopper, Warramaba virgo and its Bisexual Relatives. IX The Ribosomal RNA Cistrons.


Appendix Table 1A

This Table represents three of the best known classifications of snakes that have been proposed. Though they each subdivide the elapid differently they all agree on an advanced position of this group among snakes. The most recently proposed classification, that of Smith et al., depends heavily on the works of McDowell. In doing so, however, the author's have not clearly stated those genera and species composing the tribes in the family elapidae.
**APPENDIX TABLE 1A**

**A SUMMARY OF THE HIGHER CLASSIFICATION OF SNAKES**

Order Squamata

**Suborder Serpentes**

<table>
<thead>
<tr>
<th>Super Family Typhlopoidae</th>
<th>Infraorder Scelopodida</th>
<th>Family Typhlophidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Typhlopidae</td>
<td>Family Leptotyphlopidae</td>
<td></td>
</tr>
<tr>
<td>Family Leptotyphlopidae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Super Family Booidae</th>
<th>Infraorder Hemiphidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Anilidae</td>
<td>Family Uropeltidae</td>
</tr>
<tr>
<td>Family Uropeltidae</td>
<td>Family Xenopeltidae</td>
</tr>
<tr>
<td>Family Acrochordida</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Superfamily Colubroidea</th>
<th>Infraorder Caenophidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Colubridae</td>
<td>Family Colubridae</td>
</tr>
<tr>
<td>(28 subfamilies)</td>
<td></td>
</tr>
</tbody>
</table>

| Family Elapidae all       | Series Proteroglyphia  |
| Sub Family Apistocalaminae| Family Elapidae        |
| Stem Elapida              |                        |
| Sub Family Elapida        | Tribe Elapini          |
| Terrestrial Elapida       | Tribe Maricorini       |
| Sub Family Laticaudine    | Tribe Laticaudini      |
| Recent Sea Snakes         | Family Hydrophidae     |
| Sub Family Hydrophinae    |                        |

| Family Viperidae          | Family Viperidae       |

| Underwood 1967            |                       |
| Dowling 1967              | Smith et al., 1977    |

**Infraorder Scolecophidia**

Family Typhlophidae

Family Leptotyphlopidae

Family Anomalepididae

Infraorder Alethinophidia

Superfamily Aniloidae

Family Anilidae

Family Uropeltidae

Family Xenopeltidae

Family Acrochordida

Infraorder Caenophidia

Family Colubridae

(28 subfamilies)

Series Proteroglyphia

Family Elapidae

Family Catrictidae

Sub Family Elapidae all

front fanged

Sub Family Apistocalaminae

Terrestrial Elapidae

Sub Family Laticaudine

Recent Sea Snakes

Sub Family Hydrophinae

Sub Family Hydrophinae

Sub Family Eiphagidae

Sub Family Hydrophinae

Sub Family Hydrophinae

Sub Family Hydrophinae

Sub Family Eiphagidae

Sub Family Hydrophinae

Series Solenoglypha

Family Viperidae

Family Viperidae

Family Viperidae

Family Viperidae

Family Viperidae

Blind Snakes

Relictual Protoboids

False Coral Snakes

Pipe and Shield-tailed Snakes

Sunbeam Snakes

Mexican Python

Boas and Pythons

Wart Snakes

Pseudocrocodiloid Boas

Splitjawed Boas

Advanced Snakes

Fixed Front Fanged Snakes

Palatine Erectors

Cobras and Kraits

Asiatic Cobras (Kraits)

Afroasian Cobras

Coral Snakes

American-North Asiatic Coral Snake

South Asiatic Coral Snakes

Pelatine Dragger

Terrestrial Palatine Dragger

Vermicellines

Glyphodontines

Acanthophines

Aptostocalaines

Pseudomajines

Oxyuranines

Pseudochelines

Demainines

True Snakes

Eghalophines

Hydrelapines

Aipysurines

Hydrophines

Moveable Front-fanged Snakes
Appendix Table 1B

In the central column of this Table all genera of elapids (proteroglyphs) currently recognized are listed. To the right are listed the common names wherever possible while on the left additional comments from the literature and the relationship to the tribal categories of Smith et al., 1972 are provided.
Family: Ascomycota, Basidiomycota - Fungi

Order:Operasporales - Ascomycota

Family: Exserohilales - Basidiomycota

Genus: Exserohilum - Basidiomycota

Species: Exserohilum [species name]

Description: [Description of the species, habitat, etc.]

Notes: [Additional notes on the species, conservation status, etc.]

[Additional information on the family, order, and genus as needed]

---

Family: Basidiomycota - Fungi

Order:Entophthorales - Basidiomycota

Family: Entomophthoraceae - Basidiomycota

Genus: Entomophthora - Basidiomycota

Species: Entomophthora [species name]

Description: [Description of the species, habitat, etc.]

Notes: [Additional notes on the species, conservation status, etc.]

[Additional information on the family, order, and genus as needed]