CHAPTER I

SOMATIC AND MALE MEIOTIC CHROMOSOMES OF
THE PALE HEDGEHOG, PARAECHINUS MICROPUS BLYTH

The year 1956 was a milestone in cytology, for it was then that the investigators Tjio and Levan achieved the first accurate count of human chromosomes and developed a technique that opened up a new era in chromosomal investigations. Since then several have modified and improved chromosome preparation techniques (Rothfels and Siminovitch, 1958; Tjio and Wang, 1961) which have helped determination of diploid number, morphology and behaviour of chromosomes in many groups of animals especially the mammals. Such studies are of great significance in cytological and evolutionary analysis of various groups as in the genus Peromyscus (Hsu and Arrighi, 1968) and the genus Tupaia (Hsu and Johnson, 1963; Klinger, 1963). The chromosomal analysis has also revealed the true taxonomic relations of different species.

Hedgehogs are small sized nocturnal insectivores distributed over Europe, Asia and Africa (Ellerman and Morrison-Scott, 1966). The family Erinaceidae includes true or spiny hedgehogs. The karyological studies of Bovey (1949), Geisler and Gropp (1967), Kral (1967), Gropp (1969), Gropp et al.
(1969), Sharma et al. (1970; 1975) and Natarajan and Gropp (1971) include three genera: Erinaceus, Aethechinus and Hemiechinus. The desert hedgehogs, Paraechinus includes three species among which only Paraechinus micropus Blyth (the pale hedgehog) is found distributed in the arid regions of India. So far, no karyotypic data on the genus Paraechinus are available. In the present chapter observations on mitotic and meiotic (male) chromosomes of the pale hedgehog, Paraechinus micropus Blyth are presented.

MATERIALS AND METHODS

Animals:

Some of the hedgehogs used in the present study were collected from the fields near our University campus and some were purchased from the local animals dealers. A total of 15 animals (7 males and 8 females) were used. One of the 15 animals was identified as Paraechinus micropus Blyth, family Erinaeceidae by the Zoological Survey of India, Calcutta. The skulls and the skins of the other animals used are preserved in the laboratory and they are all identified as Paraechinus micropus (Blyth).

Bone marrow chromosomes:

Direct bone marrow chromosomes were obtained using the standard air drying technique (Rothfels and Siminovitch, 1958). Animals were injected intraperitonially with 0.02% colchicine according to their body weight (0.2ml per 100gm body weight)
2 hours prior to the sacrifice. The marrow from the humerus bones was aspirated with hypodermic syringe into a prewarmed (37°C) 0.56% potassium chloride hypotonic solution. After the hypotonic treatment of 20 minutes, the cells were fixed with 3:1 methanol acetic acid. Different variations with hypotonic solution (eg., 0.9% sodium citrate, half strength Hanks solution) at various temperatures were used, to obtain improved morphology of the chromosomes.

**Kidney culture chromosomes:**

Kidneys from adult hedgehogs were minced in Ca++ and Mg++ free phosphate buffer solution (CMF-PBS) and were trypsinized with 0.25% trypsin (Difco 1:250) in CMF-PBS. The cells were washed and finally suspended in growth medium TC 199 (Wellcome Reagents Ltd., U.K.) with 20% bovine serum with 100 units of benzylpenicillin (Alembic Chemical Works Co. Ltd.) and 50µg of streptomycin sulphate (Indian Drugs and Pharmaceuticals Ltd.) per milliliter of the medium. Ten ml of cell suspension with approximately 4-5 x 10^6 cells per ml were inoculated into each milk-dilution bottle and were grown as monolayers. The medium was renewed when the cultures were half confluent. As a routine the cultures were harvested after 48 hours of medium renewal. Cultures were treated with 0.1µg colchicine per ml for final 2 hours before harvesting. The cells were loosened by mild trypsinization and treated with half strength growth medium as hypotonic solution for 15 minutes at room temperature (25-27°C). The material was fixed with methanol acetic acid (3:1).
Slide preparation:

For both the material from bone marrow and kidney cultures, the fixative was changed at least thrice before preparing the slides. Slides were prepared by keeping a few drops of cell suspension in fresh fixative on precleaned and wet slides and were allowed to dry at room temperature. The slides were either stained with carbol fuchsin or Giemsa.

Meiotic preparations:

Meiotic chromosome preparations were made from testicular material by the method of Evans et al. (1964) and Ford and Evans (1969). Testicular tubules were liberated in 2.2% sodium citrate solution and were washed repeatedly to remove the fat. The tubules were minced in fresh solution of 2.2% sodium citrate solution and were mixed by repeated pipetting with a long nozzle dropper for 5 minutes. The supernatant (avoiding the pieces of tubules) was centrifuged and the cells were treated with 1% sodium citrate hypotonic solution for 10 minutes at room temperature. The material was fixed with methanol acetic acid (3:1). The slides were prepared and stained as described above for the mitotic chromosomes.

For squash preparations of testis, the material was fixed in methanol acetic acid without any hypotonic pretreatment. The tubules were stained with 0.1% aceto-orcein for 5 minutes and the material was squashed under the thumb pressure.

Photographs were taken on Reichert Zetopan Photomicroscope with bright-field illumination under an oil immersion
TABLE I

Frequency distribution of chromosome numbers of the pale hedgehog, *Paraechinus micropus* Blyth

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total no. of cells counted</th>
<th>Chromosome number</th>
<th>% of cells with 48 chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>
lense at an initial magnification of 500x. A high contrast copying film (ORWO) DK-5 was used.

**OBSERVATIONS**

Chromosomes from both bone marrow and kidney cultures were used for counting the diploid number and karyotyping. However, the chromosomes obtained from the bone marrow gave very poor morphology even after repeated attempts with various variations of hypotonic solutions, different temperatures and fixative. Hence, for the morphological studies of chromosomes only kidney culture cells were used.

Well spread metaphases, which appeared to be complete were selectively used for counting the diploid chromosome number. Chromosomal counts from more than 100 metaphases from both the sexes revealed the diploid number of 48. Counts varied little from 46 to 48 with 93 percent of cells showing 48 chromosomes (Table I).

All the individuals show identical karyotypes. The metaphases and the karyotypes of male and female hedgehogs are shown in figures 1 to 4. The autosomal complement of the karyotype consists of a pair of large subtelocentrics (ST\(_1\)), seven pairs of graded telocentrics (T\(_1\)–T\(_7\)), thirteen pairs of large to small sized meta- and submetacentrics (M\(_1\)–M\(_{13}\)), and two pairs of very small chromosomes (probably submetacentrics). Of the sex chromosomes, X chromosome is the second largest metacentric and the Y chromosome is the smallest metacentric nearly the size of the smallest autosomes.
TABLE II

Morphometric data of chromosomes of hedgehog, Paraechinus micropus Blyth from kidney culture cells (an average of 5 male and 5 female karyotypes)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chromosome type &amp; No.</th>
<th>Mean percentage haploid complement length</th>
<th>Mean arm ratio L/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ST₁</td>
<td>7.84</td>
<td>3.38</td>
</tr>
<tr>
<td>2</td>
<td>T₁</td>
<td>6.43</td>
<td>..</td>
</tr>
<tr>
<td>3</td>
<td>T₂</td>
<td>4.69</td>
<td>..</td>
</tr>
<tr>
<td>4</td>
<td>T₃</td>
<td>4.05</td>
<td>..</td>
</tr>
<tr>
<td>5</td>
<td>T₄</td>
<td>3.42</td>
<td>..</td>
</tr>
<tr>
<td>6</td>
<td>T₅</td>
<td>3.05</td>
<td>..</td>
</tr>
<tr>
<td>7</td>
<td>T₆</td>
<td>2.32</td>
<td>..</td>
</tr>
<tr>
<td>8</td>
<td>T₇</td>
<td>1.78</td>
<td>..</td>
</tr>
<tr>
<td>9</td>
<td>M₁</td>
<td>7.59</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>M₂</td>
<td>6.76</td>
<td>1.05</td>
</tr>
<tr>
<td>11</td>
<td>M₃</td>
<td>5.58</td>
<td>1.05</td>
</tr>
<tr>
<td>12</td>
<td>M₄</td>
<td>5.48</td>
<td>1.16</td>
</tr>
</tbody>
</table>

(Contd..)
TABLE II (Contd..)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chromosome type &amp; No.</th>
<th>Mean percentage haploid complement length</th>
<th>Mean arm ratio L/S</th>
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<tbody>
<tr>
<td>13</td>
<td>M_5</td>
<td>5.06</td>
<td>1.40</td>
</tr>
<tr>
<td>14</td>
<td>M_6</td>
<td>4.42</td>
<td>1.21</td>
</tr>
<tr>
<td>15</td>
<td>M_7</td>
<td>4.25</td>
<td>1.08</td>
</tr>
<tr>
<td>16</td>
<td>M_8</td>
<td>4.21</td>
<td>1.50</td>
</tr>
<tr>
<td>17</td>
<td>M_9</td>
<td>3.37</td>
<td>1.66</td>
</tr>
<tr>
<td>18</td>
<td>M_{10}</td>
<td>3.05</td>
<td>1.41</td>
</tr>
<tr>
<td>19</td>
<td>M_{11}</td>
<td>2.63</td>
<td>2.12</td>
</tr>
<tr>
<td>20</td>
<td>M_{12}</td>
<td>2.53</td>
<td>1.00</td>
</tr>
<tr>
<td>21</td>
<td>M_{13}</td>
<td>1.89</td>
<td>1.25</td>
</tr>
<tr>
<td>22</td>
<td>S_1</td>
<td>1.26</td>
<td>..</td>
</tr>
<tr>
<td>23</td>
<td>S_2</td>
<td>0.84</td>
<td>..</td>
</tr>
<tr>
<td>24</td>
<td>X</td>
<td>6.11</td>
<td>1.07</td>
</tr>
<tr>
<td>25</td>
<td>Y</td>
<td>1.26</td>
<td>..</td>
</tr>
</tbody>
</table>

ST = subtelocentric, T = telocentric, M = meta- and submeta-centric, and S = small chromosomes.
The telocentric chromosomes show weak centromeric regions usually with both the chromatids separate. The three small telocentric pairs ($T_5-T_7$) show the presence of satellites (figs. 2 and 4). The normal chromosomes from kidney cultures $M_2$, $M_7$ and the X from all the karyotypes show a characteristic Giemsa staining pattern. The pericentric regions except for the centromere show faint or no staining (fig. 5). The chromosome pair $M_3$ shows considerable size difference between the two homologues with light staining region on the longer homologue. The pair $M_4$ has unstained region with Giemsa on the short arm close to the centromere (fig. 5). The NF (nombre fundamentale of Matthey, 1945) could be determined as 82 in Paraechinus microps.

The idiogram presenting relative lengths and centromeric positions is shown in figure six. The morphometric data is presented in Table II. The X chromosome is normal mammalian type making 6.11% of the haploid complement. The Y chromosome is very small and is only 1.26% of the haploid complement.

The interphase nuclei from kidney culture cells from male and female show many chromocenters formed probably from autosomal heterochromatin. This obscures a precise identification of sex chromatin in female cells.

**Meiosis in male:**

The spermatogonial divisions from testes typically show normal diploid chromosomes 48 (fig. 7). At pachytene the heterochromatin is present as two to three prominent blocks.
### TABLE III

Frequency of X/Y bivalents and univalents at metaphase I of meiosis in hedgehogs

<table>
<thead>
<tr>
<th>No. of plates</th>
<th>X/Y bivalents</th>
<th>%</th>
<th>X/Y univalents</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>172</td>
<td>148</td>
<td>86.1</td>
<td>24</td>
<td>13.9</td>
</tr>
</tbody>
</table>

### TABLE IV

Frequency of normal and polyploid germ cells observed at different stages of meiosis in male hedgehog (*Paraechinus micropus* Blyth)

<table>
<thead>
<tr>
<th>Division</th>
<th>n</th>
<th>2n</th>
<th>3n</th>
<th>4n</th>
<th>6n</th>
<th>% normal divisions</th>
<th>% abnormal divisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatogonial</td>
<td>22</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>89.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Metaphase I</td>
<td>323</td>
<td></td>
<td>35</td>
<td>14</td>
<td></td>
<td>86.80</td>
<td>13.10</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>206</td>
<td>70</td>
<td>24</td>
<td></td>
<td></td>
<td>68.60</td>
<td>31.30</td>
</tr>
</tbody>
</table>
The sex chromosomes precociously condense and form heteropycnotic sex vesicle, as in many other mammals (fig. 8). The autosomes and sex chromosomes form a total of 24 bivalents in majority of the meiotic metaphase I (fig. 9). The sex bivalent is formed by characteristic terminal association of Y to one of the arms of the X chromosome and the bivalent XY is generally heteropycnotic. In about 14% of the metaphase I divisions the X and Y do not appear as bivalents but remain as univalents (Table III and fig. 10). Rarely, in some cells, the smallest autosomes also occur as univalents. After reduction, the metaphase II normally showed n=24 chromosomes with either the X or Y chromosome (figs. 11 and 12). In less condensed division figures the chromatids are diverged apart and show characteristic spiral configuration. On the other hand, in condensed chromosomes the spiral structure becomes obscure. The sex chromosomes X and Y are not heteropycnotic at metaphase II.

The meiotic preparations of the hedgehog, *Paraechinus micropus* showed a significantly high percentage of polyploid cells at different meiotic stages. These polyploid cells were seen in air dried as well as squashed preparations. Table IV shows the percentage of polyploid cells at different stages. They are 11 percent at spermatogonial divisions, 13.1 percent at meiotic metaphase I (fig. 13) and 31.3 percent at meiotic metaphase II (fig. 14). Among the polyploid cells at metaphase I, some cells were seen different chromosome sets in the same plate differing in the degree of condensation (figs. 15 and 16). This variation could be best seen in terminally associated XY bivalents, hence they are shown separately for an easy comparison.
The pale hedgehog, *Paraechinus micropus* Blyth, has diploid chromosome number of 48 like other hedgehog species studied, *Erinaceus europaeus* (Bovey, 1949; Geisler and Gropp, 1967; Gropp et al., 1969), *Erinaceus roumanicus* (Geisler and Gropp, 1967; Kral, 1967), *Hemiechinus megalotis* and *H. auritus aegiptius* (Gropp, 1969; Gropp et al., 1969) and *Hemiechinus auritus collaris* (Sharma et al., 1970; 1975). Regardless the constant chromosome number of 48 in most of the genera, except in the case of *E. amurensis* where the chromosome number is 44 (Kang and Kim, 1963), there are considerable karyotypic variations. The karyotypes of the genera *Paraechinus* (Shah et al., 1975), *Hemiechinus, Erinaceus* and *Aethechinus* show some similarity in the large number of meta- and submetacentrics and at least two pairs of very small chromosomes with an exception of *Erinaceus* (*Aethechinus*) *algirus* *lavaudeni* (Gropp and Natarajan, 1972) which has three pairs. The main differences in the karyotypes are due to the differences in the fundamental number (NF of Matthey, 1945) of 82 in *Paraechinus micropus* in contrast to the high number of 92 in *E. europaeus europaeus* and in *E. concolor roumanicus* (Kral, 1967). The *Paraechinus* stands distinct with a graded series of large number of telocentrics (7 pairs) which is totally absent in *Hemiechinus* and *Aethechinus* and is represented by only one pair in *Erinaceus* species. However, in the later three genera there is instead a large number of meta- and submetacentrics, thus maintaining the constant number of 48 chromosomes in the family *Erinaeceaidea*. 
These changes may be attributed to various pericentric inversions as also suggested by Gropp (1969).

In *Erinaceus europaeus*, there are three large blocks of heterochromatin which are negatively pycnotic in normal Giemsa staining (Citoler et al., 1972). Similar diffuse Giemsa staining blocks are present in *E. (Aethechinus) algirus* (Gropp and Natarajan, 1972) but are two only in number. In *P. micropus* (present study) and *Hemiechinus megalotis* and *H. auritus* (Gropp et al., 1969) such non-centromeric or terminal blocks are absent. However, similar diffuse, Giemsa negative regions are present in *Paraechinus micropus* as small blocks on two of the metacentric autosomal pairs (*M₂* and *M₇*) and the X chromosome near the centromeric regions. These are late replicating and C-band positive (Chapter II and III) similar to those of *Erinaceus* and *Aethechinus* species (Gropp and Natarajan, 1972; Citoler et al., 1972). The prominent features of *P. micropus* are the heteromorphism in the size of the medium sized metacentric (*M₃*) and a prominent Giemsa negative region on metacentric pair (*M₄*).

From the testicular preparations it was shown that in European and Algerian hedgehogs the large heterochromatic segments separate prematurely during diakinesis (Natarajan and Gropp, 1971). But in the case of the pale hedgehog, the smallness of the heterochromatic segments obscures clear observations. However, the nuclear heterochromatin forms two to three blocks at pachytene.
During meiotic division all the autosomes and sex chromosomes of hedgehog form bivalents in most of the cases. The X and Y chromosomes form bivalent by a characteristic terminal association and is very similar to the other hedgehogs. Though, this terminal association of X and Y in human males is known since long (Painter, 1923), the possibility of occurrence of chiasma in sex bivalents has been discussed by many (Ford and Hamerton, 1956; Book and Kjessler, 1964; Sasaki and Makino, 1965; Fredga and Santesson, 1965). In some mammalian species with rather large Y chromosome, the sex bivalent forms chiasma at interstitial regions which is possibly autosomal in origin and ends up in the terminal association (Hamerton, 1958; Ohno and Weiler, 1961). Further, with the studies of electron microscope on mouse sex vesicle, Solari (1964) reported the presence of synaptonemal complex showing the possibility of crossover in the terminal association but failed to show in man (Solari and Tres, 1967). The present study could not provide any evidence of chiasma formation in hedgehog.

The sex chromosomes X and Y occur as univalents in 13.9% of the cells at diakinesis and metaphase I in hedgehog. Similar univalents were reported in human males by Ford and Hamerton (1956), Sasaki and Makino (1965), Sasaki (1965) and Kjessler (1966). In human males the reported frequencies of univalents varied markedly from 4.4 percent (Kjessler, 1966) to 31.2 percent (Sasaki, 1965). It was suggested by Ford and Hamerton (1956) that the more number of univalents were seen with more vigorous spreading techniques. To an extent this
seems possible at least in the case of hedgehog, as the metaphase I figures near the edge of the slide have more of univalents of sex chromosomes as well as small autosomes than those in the middle of the slide. But, this observation can not completely deny the occurrence of sex chromosomal univalents due to the failure of synapsis in the natural cell population.

At metaphase II the chromatids are drawn apart and show coiled nature. The heteropycnotic sex chromosomes described by Makino and Sasaki (1961) and Ohno (1962) are not seen in hedgehog meiotic metaphase II.

Polyploids in germ cells have been reported in man and other mammals (Darlington and Haque, 1962; Sasaki, 1965; Sasaki and Makino, 1965; McIlree et al., 1966). In hedgehog also the polyploids were seen at all division stages, viz., 11 percent at spermatogonial, 13.1 percent at diakinesis plus metaphase I, and 31.3 percent at metaphase II. Since the polyploid cells at various division stages are also seen in squash preparation of hedgehog testis (without hypotonic pretreatment), it is unlikely that the polyploid meiotic cells seen in air dried preparation are artifacts of the technique. The significance of some of the polyploid cells at metaphase I with differential condensation of different chromosome sets is not clear.

Darlington and Haque (1962) reported that the polyploid germ cells in mouse do not mature into sperms due to high fragmentation of secondary spermatocytes. On the other hand, Sasaki and Makino (1965) observed giant sperm heads in
human males. However, in hedgehog, the fragmentation of secondary spermatocytes was seen rarely but their frequency cannot make up the number of polyploids observed.

Many human abortuses are hyperdiploid including triploids and tetraploids (Carr, 1963; 1965; Geneva Conference, 1966). Such polyploid embryos are reported in other mammals like rat and mouse. Though there is some evidence that the polyspermy and failure of reduction in ovum contribute to form polyploid zygotes (Austin and Bishop, 1957), it is not known whether the polyploid germ cells contribute by forming mature hyperploid sperms, in the origin of polyploid embryos.

In conclusion, the *Paraechinus micropus* Blyth shows some karyotypic similarity particularly the heterochromatic segments with the other hedgehog genera studied, but stands distinct from *Erinaceus*, *Aethechinus* and *Hemiechinus* in its other karyotypic characters. The meiotic behavior is similar with other hedgehogs reported, however, polyploid cells have not been reported in other hedgehogs.

**SUMMARY**

The pale hedgehog, *Paraechinus micropus* Blyth, has diploid chromosome number of 48 in both the sexes. The autosomal complement consists of a single pair of subtelocentrics, seven pairs of graded telocentrics, thirteen pairs of large to small meta- and submetacentrics, and two pairs of extremely
small chromosomes (microchromosomes). X chromosome is a medium sized metacentric, and Y chromosome is nearly the size of the smallest autosomes. The telocentric chromosomes show weak centromeric regions. The last three pairs of telocentrics bear satellites. The second and seventh metacentric pairs and X chromosome show dull stained regions on both the arms on either side of the centromere. The fourth metacentric pair has a prominent secondary constriction on the short arm near the centromere. The X chromosome is a little more than the "original type", making 6.8% of the total haploid complement. The sex determining mechanism is the usual mammalian type, with XX female and XY male.

In testicular meiotic divisions, in general, all the chromosomes form 24 bivalents. However, in about 14 percent meiotic metaphase I, the X and Y chromosomes occur as univalents. At pachytene heterochromatic segments of different chromosomes form two to three chromocenters. The testicular preparations from male hedgehogs showed a considerable incidence of polyploid germ cells during all the division phases, 11 percent at spermatogonial, 13.1 percent at metaphase I and 31.3 percent at metaphase II divisions. The significance of polyploid occurrence is discussed.
REFERENCES


Fig. 1. Metaphase plate from kidney culture of a male hedgehog, *Paraechinus micropus* Blyth (stained with Giemsa).

Fig. 2. Karyotype of male hedgehog, *P. micropus* showing a pair of large subtelocentrics (ST\textsubscript{1}), 7 pairs of telocentrics (T\textsubscript{1}-T\textsubscript{7}), 13 pairs of graded meta- and submetacentrics (M\textsubscript{1}-M\textsubscript{13}) and 2 pairs of small submetacentric chromosomes (S\textsubscript{1}&S\textsubscript{2}). X is the larger metacentric and Y is small and is about the size of S\textsubscript{1}. 
Fig. 3. Metaphase chromosomes of female hedgehog, *Paraechinus micropus* Blyth (from kidney cultures and stained with Giemsa).

Fig. 4. Karyotype of female hedgehog, *P. micropus*

- ST<sub>1</sub> = subtelocentrics
- T<sub>1</sub>-T<sub>7</sub> = telocentrics
- M<sub>1</sub>-M<sub>13</sub> = meta- and submetacentrics
- S<sub>1</sub>-S<sub>2</sub> = small submetacentrics.
Fig. 5. The chromosomes $M_2$, $M_7$, X and $M_4$ of the pale hedgehog, *P. micropus* from male (A) and female (B) diploid metaphase cells and from a male (C) tetraploid cell from primary kidney cultures. Stained with Giemsa. Note the pericentric regions on the chromosome pairs $M_2$, $M_7$ and the X showing dull or no staining. The chromosome $M_4$ showing a constriction with variant staining properties near the centromere.
PLATE: IV

Fig. 6. The idiogram showing the karyotype of hedgehog, *Paraechinus micropus* Blyth. The idiogram presents the arm ratios, actual length, relative percentage and the chromosome number.
PLATE: V

Fig. 7. A spermatogonial division with 48 chromosomes from testicular preparations of hedgehog, *P. micropus* (air dried preparation and Carbol fuchsin stain).

Fig. 8. A pachytene stage from testes of hedgehog showing heterochromatin segments and sex vesicle (air dried preparation and Giemsa stain).
PLATE: VI

Fig. 9. A meiotic metaphase I complement from the male hedgehog showing 24 bivalents. Arrow shows the terminally associated XY bivalent (air dried preparation and carbol fuchsin stain).

Fig. 10. A metaphase I spread from testes of hedgehog, *P. micropus* showing 23 bivalents and XY univalents (preparations are same as above).
PLATE: VII

Fig. 11. A meiotic metaphase II from a male hedgehog, *P. micropus*, showing haploid chromosomes set with Y chromosome.

Fig. 12. A meiotic metaphase II from a male hedgehog, with X chromosome.

(Both are from air dried preparations and stained with carbol fuchsin).
PLATE: VIII

Fig. 13. A tetraploid metaphase I from testes of hedgehog, *P. micropus*. One of the sex chromosome pair is seen as bivalent and the other as univalents.

Fig. 14. A hexaploid metaphase I from testes of hedgehog, *P. micropus*. 
PLATE: IX

Fig. 15. A tetraploid metaphase I from testes of hedgehog, P. micropus showing differential condensation. The XY bivalents are shown below separately for comparison. (air dried preparation and carbol fuchsin stain).

Fig. 16. A hexaploid cell at metaphase I. Details are same as above.