2004

Cytogenetical Studies of Some Ladybird Beetles (Coleoptera: Coccinellidae) of Bangladesh

Das, Rina Rani

University of Rajshahi

CYTOGENETICAL STUDIES OF SOME LADYBIRD BEETLES (COLEOPTERA: COCCINELLIDAE) OF BANGLADESH

Thesis Submitted for The Degree
of
Doctor of Philosophy
in the
Institute of Biological Science Rajshahi University
by
Mrs. Rina Rani Das

June, 2004

Integrated Pest Management Laboratory
Institute of Biological Science
Rajshahi University
Rajshahi 6205
Bangladesh

Dedicated
to
my son

DECLARATION

This thesis contains no material which has been submitted for the award of any other degree or diploma in any university. To the best of my knowledge, it contains no previously published or written by any other person except when due reference is made in the text of the thesis.

29.06.04

June 2004 Mrs. Rina Rani Das

CERTIFICATE

This is to certify that the thesis entitled "CYTOGENETICAL STUDIES OF SOME LADYBIRD BEETLES (COLEOPTERA: COCCINELLIDAE) OF BANGLADESH" Submitted for the degree of doctor of philosophy, is the outcome of bonafide and original research work of Mrs. Rina Rani Das.

June, 2004.

Md. Sohrab Ali 30.6.2004
Supervisor
Professor
Department of Zoology
Rajshahi University
Rajshahi, Bangladesh.

CONTENTS

Acknowledgement

I would wish to express my most humble indebtedness and my profound sense of gratitude to my respectable supervisor, professor Md. Sohrab Ali; Department of Zoology as well as the honorable previous Director, Institute of Biological Sciences (IBSc.), Rajshahi University, for his scholastic guidence. I feel beholden and cherish my appreciation for his incisive interest that led me through many difficult and perplexing technical labyrinths. His valuable suggestions, stimulating discussions and editorial insight had been my greatest support in compelling this dissertation. Grateful thanks are due to the respectable Chairman and members of the selection board of fellowship 1998-'99 session, IBSc. Rajshahi University for offering me a junior research fellowship and to the Ministry of Education, Government of the Peoples Republic of Bangladesh for granting me deputation while conducting this Research.

I gratefully acknowledge to the respectable professor of IBSc. and present pro vice-Chancellor of Rajshahi University, Dr. K. A. M. Shahadat Hossain Mandol Dr. M. A. Bari, professor and honourable present Dirrector of IBSc., Dr. Wahidul Islam, professor and Dr. Parvez Hassan, Research fellow, IBSc. for their cooperation as needed.

I avail myself of the opportunity, gratefully acknowledge to Dr. M. Saidul Islam, Chief Scientific Officer, Institute of Food & Radiation Biology, Atomic Energy Commission, Savar, Dhaka and professor Dr. Jalal Uddin Akbar, Principal (Rtd.) of Rajshahi Govt. College, Rajshahi; for their tremendous help in different corners of my research. I should like to express my gratitude to Dr. Md. Sayedur Rahaman, professor (Rtd.) of the Dept.of zoology, Rajshahi University; Dr.M. A. Mannan, Dr. Saiful Islam, Dr. A. K. Saha and Dr. Mahbub Hassan, professors of the dept. of Zoology. Rajshahi University for their valuable suggestion and encouragement.

My deepest appreciation is extended to Dr. Bidhan Chandra Das, prof. of Zoology Rajshahi University for his wide help to communicate the authoress with the scholar experts of the Universities of Kalyani and Kolkata, India.

I express deep sense of gratitude to Dr. Chitta Ranjan Sahu, Reader, and dept. of Zoology, and Dr. Anil Kumar Saha, prof., dept. of Zoology, Kalyani University, India for their academic suggestion. Some of their published papers were very helpful for my research work.

I gratefully acknowledge to Nobuo Tsurusaki, Susumu Nakano and Haruo Katakura, Division of Biological Sciences, Graduate School of Science, Hokkaido, Japan for their important papers and theoretical guide-line of chromosomal technique, which helped me in a un-parallel way. I am also grateful Dr. Aktaruzzaman, professor of Botany, Dhaka University for his some important words related to my project. It is also my gratitude to Dr. Golam Kabir, Professor and Chairman of Agronomy, Rajshahi University and Dr. Manjur Hossain, professor, dept. of Botany, Rajshahi University for their scholastic advice in my research work.

I avail myself of the opportunity to gratefully acknowledge the debt for the tremendous amount of generous support and kind assistance of Dr. Azizul Islam and Dr. Basudeb Das, professors of the Dept. of Chemistry, Rajshahi University who had allowed me in their laboratory to do the work on TLC. Without which it was very hard to complete a part of my research.

I would like to express sincere gratitude to Dr. Abdul Khaleque, professor & the previous Chairman of the Dept. of Genetics and Breeding for allowing me to use their micro-photographic camera. I am also thankful to other teachers of the Dept. of Genetics & Breeding to help me in micro-photographic works.

My deepest sense of gratitude is extended to Dr. M. Altaf Hossain, prof., Dept. of Zoology, Rajshahi University for his strong moral support in my project.

I would wish to express my sincerest thanks to the learned members of the Dept. of Zoology, Rajshahi University who had eased my labor immeasurably by extending friendly and strong moral support, which afforded me a sturdy working foundation.

The realization of my long cherished dream would have been very remote without the scholastic help from many great scholars both from home and abroad who gave benefit of their knowledge, which contributed to the success of this work.

I do remember with great reverence Prof. Haruo Katakura, Division Biological Sciences, Graduate School of Science, Hakkaoido University, Japan; Professor Miss Amita Chakrabarti, Dept. of Science of life, Kolkata University, Professor G. P. Sharma and Professor O. P. Mittal of the University of Punjab, Chandigarh, India; Prof. A. K. Saha, Dept. of Zoology, Kalyani University, Kolkata, India. ; Dr. Saidul Islam, Chief Scientific Officer, Institute of Food and Radiation Biology, Atomic Energy Commission, Savar Dhaka for their generous contribution of specialized knowledge by kindly forwarding a great deal of cytogenetical literatures which have gone a long way to upgrade this dissertation. I am grateful to the officers of BANSDOC for procuring me some rare documents (papers) from overseas. My deepest appreciation is extended to my colleagues for their co-operation and alround help.

Grateful acknowledgement is specially extended to K.F.M. Mazharul Haque &, Dr. Asfar Ahmed, Associate Professors in Zoology, Carmicheal College, Rangpur, Dr. Jasibar Rahman, Asst. prof. in Zoology, Dinajpur Govt. College, Dinajpur, for their occasional help. This work would have been impossible without the splendid co-operation and spontaneous assistance of so many persons that it is impossible to give proper and individual credit to all of them. To name all those to whom I am indebted in some way or be gracious enough to accept this blanket of gratitude. I however remember with affection Mr.Kamrul Hassan who puts his youthful generosity into this painstaking endeavor. Thanks are due to the fellow of the IBSc. Rajshahi University. Whose love and support would ever remain enshrined in my memory? I have also the pleasure to express my appreciation and thanks to all officials and staff of the IBSc, Rajshahi University for collaborative contributions during research period. It is duty not less than a pleasure to make a special acknowledgement to extend my obligation, many of my relatives, well wishers. I am especially grateful to my Son and my Husband for their help during the collection of the ladybird beetles and also for their sacrifice during the endeavor.

This work would have many more blemishes without the extensive researchers in this field by many previous workers from whose literatures I have drawn freely. I wish to express my deepest sense of gratitude for the source and use of ideas statistically and scientifically. Any mistake, misrepresentation or omission is entirely of my own. I apologies to many whose works may not have been cited or acknowledged.

The Authoress.

Abstract

Twenty species of lady beetles, *Rodalia fulvescens* Hoang, *Sterthorus tetranychi* Kapur, *Scymnus nubilus* Mulsant, *Brumoides lineatus*,(Weise), *Jauravia pallidula* Motchulsky., *Pharoscymnus taoi* Sasaji, *Illeis indica* Timberlake, *Psyllobora bisoctonotata* (Mulsant), *Cheilomenes sexmaculata* (Fabricius), *Coccinella septempunctata* Linneaus, *Coccinella transversalis* Fabricius, *Harmonia octomaculata* (Fabricius), *Micraspis discolor* Fabricius, *Micraspis yasumatsui* Sasaji, *propylea quatuordecimpunctata* Linneaus, *Apomicraspis quayumi* Ali & Rahman, *Afidenta misera* Mulsant, *Epilachna septima* (Mulsant), *Epilachna pusillanima* (Mulsant) *and Epilachna vigintioctopunctata* (Fabricius), belonging to 16 genera of the six sub families; Coccidulinae, Scymninae, Chilocorinae, Sticholotidinae, Coccinellinae and Epilachninae under the family Coccinellidae of Coleoptera of Bangladesh have been cytogenetically investigated. Detailed comparison of karyotype using principal components analyses revealed a considerable divergence among the 20 species. Sex determining mechanism is common with a large X chromosome and a minute y chromosome in a nonhomologous formation. The lowest number of chromosomes obtained 2n=14 in present investigation from the spematogonial cell of *Scymnus nubilus* and *Afidenta mesera* with the karyotype fomula 6AA+Xy. The species *Harmonia octomaculata and E pusillanima* showed 2n=16 with the Karyotype 7AA+Xy. Diploid number of chromosome in the four species *Brumoides lineatus, Jauravia pallidula, psyllobora bisoctonotata and Epilachna vigintioctopunctata* were 18 with the karyotype formula 8AA + Xy in each case. Other 12 species of present investigation have shown the modal number of chromosomes 2n=20 with the karyotype 9AA+Xy. X chromosomes were sub metacentrc in the five (5) species *Coccinella septempunctata, Harmonia octomculata, Micraspis discolor, M. yasumatsui* and *Afidenta misera.* Other 15 species showed metacentric type of X chromosomosome. Variations of centromeric position were found in autosomes of the 20 species. Of the autosomes most are metacentric type than submetaecntric and few are telocentric.

The ratio of autosomes showed a great diversity. In each case last pair of autosome showed minimum value ranged from 5.21% in *E. septima* to 9.8% in *Afidenta misera,* while 1st pair of autosome showed highest value ranging between 10.67% in *Propylea quatuordecimpunctata* to 17.10% in *Afidenta misera.* Sex chromosome ratio were highest in the total numbers of chromosomes in each species, which found highest 18.82% in *scymnus nubilus* and 18.8% in *Epilachna vigintioctopunctata* and lowest 12.18% in *Psyllobora bisoctonotata.* Chromatographical analysis in 12 coccinellids, *Illeis indica, Psyllobora bisoctonotata, cheilomenes sexmaculata, Coccinella septempunctata, Coccinella transversalis Harmonia octomaculata, Micraspis discolor, Micraspis yasumatsui, Afidenta misera,* Epilachna *septima, Epilachna pusillanima and Epilachna vigintioctopunctata* showed three amino acids alanine, lysine and glycine present in high concentration in each species. Aspartic acid and glutamic acid were absent in four species of the Epilachninea. Qualitative variations of other amino acids were found from species to species. The highest number of amino acids (11) was identified in *C. septempunctata* and *C. transversalis* and the lowest number of amino acids (8) were detected in *E. septima.* The present chromatographical analyses showed the qualitative variations in amino acid contents from species to species. So it might be assumed that the non overlapping measurements and ratios of chromosome in different species as well as the variations of amino acids composition have effect on cytogenetics of ladybird beetles.

ABBREVIATIONS AND ACRONYMS

AA	autosome pair
AR	arm ratio, (l/s) of chromosome
CI	Cetromeric Index of chromosome (s/s+l)
IBSc	Institute of Biological Sciences
IPM	Integrated pest management
TLC	Thin layer chromatography
l	long arm of chromosome
m	metacentric,
RL	Relative length of chromosome (s+l)/(∑s+1)
Ff	Relative of front
s	short arm of chromosome
sm	submetacentric type of chromosome
t	telocentrictype of chromosome,
X	sex chromosome,
XX	female sex chromosome,
Xy	with small male chromosome,
Xyp	in male Xand y chromosomes are in a form of a man hanging with a parachute
neoXY	when the Y chromosome is not distinguishable from autosome

CHAPTER – 1

INTRODUCTION

Introduction

The ladybird beetles or 'Katale-poka' are well known to the people of Bangladesh for their scientific importance and prettiness. Many ladybird beetles have bright contrasting colour pattern. Some are red with black spots, others are yellow and black or maroon spots and some have stripes instead of spots and some have no spot at all. Ladybird beetles are connected with good fortune in many myths and legends. The term 'lady' is in reference to biblical mother mary (Roache, 1960). The red colour is said to represent her clock which in early paintings and sculptures was usually depicted as being red and the seven black spots represent the seven joys and seven sorrows.

The ladybird beetles comprise a coleopteran-family Coccinellidae. Latreille (1810) for the first time established the family Coccinellidae. Linnaeus (1858) described 36 species under the genus *Coccinella* and since then about 4,500 described species of the Coccinellidae have been established worldwide (Booth, 1993). Fowler (1912) mentioned about 2,000 species in the world, while according to other sources the number of species ranges between 3,500 and 5,000 (Korschefsky, 1931; Sasaji 1968, 1971; Imms 1977; Pope 1978; Iablokoff-Khnozorian, 1979;1982; Majerus & Kearns, 1989 and Booth *et.al* 1990). Phylogenetic relationship among the family was studied by Hodek (1973).

Many species are common. They may be found in almost any habitat from sea-coast to mountain top, and from city wastelands to windswept health lands. Almost every garden will have at least one species.

Most ladybird beetles are carnivorous. Many species and aphidophagous in both adults and larval stages.The carnivorous lady bird beetles are frequently the key factors in regulating homopteran insects and phytophagous mites (Clausen, 1956 and Sweetman, 1958). So, ladybird beetles are of great importance as major natural predators of these pests. A few numbers of the Coccinellidae have been used as biocontrolling agents in USA, South Africa, Australia, Newzealand and other countries. Currently four species of ladybird mass cultured for the suppression of many crop pest (Rabindra, 2002). The main theme of integrated pest management (IPM) is to reduce the use of chemical pesticides and utilise biological, plant breeding, cropping measures etc. (Grosse-Ruschkamp, 1994). With a view to reduce the environmental pollution.

Another group of coccinellid beetles are phytophagous in habits and causes considerable damage to various cultivated plants specially belonging to the family Cucurbitaceae, Solanaceae and Papillionaceae, often they completely defoliate the plants like bitter gourd (*Momoridica charantiia* L.), Kakrole *(Momoridica cochin chinensis speng)* and causes serious damage to egg plants l *(solunum melongenal)*, Tomato (*hycopersicum esculentum* Mill) and potato *(solunum tuberosum)* and beans. A small group feeds on mildew and other fungi.

Bangladesh has an agro-based economy. The control of pests of agricultural crops, fruit-trees etc, is vital to boost up its economy. Global warming has cautioned us and the adverse consequences of pesticide use are always alarming and also inducing pest out break because of pest resistance and mass propagation. The entomological backlashes have compelled the scientists to be concerned with compatible pest management programmes. The beneficial Cocconellidae may be an alternative and complementary means to insecticidal spray.

Substantial contributions on the taxonomy of the Asian Coccinellidae were made by Kapur (1940-'73) mostly dealt with the taxonomy of Indian ladybird beetles. Taxonomic studies on the Pakistani coccinellidae were contributed by Ghani (1962), Ahmad & Ghani (1966) and Ahmad (1968, 1970, 1973).

Moreover there are lists of coccinellids by local workers like Alam (1962, 1967). Alam *et al.*(1964), Kabir (1975). Rahman *et al.*(1995). First ever species described from west Bengal is *coccinella suturalis* Fabricius =*Brumoides suturalis* (Fabricius). The species was described in1798. *Coccinella dodecastigma* Wiedman=*Epilachna dodecastigma* (Wiedman) (= *Epilachna pusillanima*) is the next species described in 1823. Mulsant (1850) in his world monograph on the coccinellidae described 7 more species namely *Rodalia fumida, Rodalia ruficllis, Coelophora biosellata, Coelophors westernmanii, Calvia dorsonotta, Epilachna grsdaria=Afidenta gnadaria.* In 1853 Mulsant added two more *species Scymnus (pullus) pyrocheilus and Cryptogonus acriasi.* Ten years latter Motschulsky (1866) added another species *Scymmus brennescens.* Thus prior to this work 60 species of Coccinellidae were known from the state of west Bengal.

Perior to Mulsants work (1846) practically there was no classification for Coccinellidae. Since than a number of workers namely Mulsant (1850). Crotch (1874), Sicard (1907), Dabzhansky (1925),Korschefky(1931) and Bielawsky (1979) contributed extensively towards the development of the classification of the family Cocconellidae. Sasaji (1967, 1971) put forward a classification in which he noted that the Coccinellidae belongs to the coleopteran super family Cucujoidea and devided whole family into six sub families namely-Sticolotinae, Chilochorinae, Scymninae, Coccidulinae, Coccinellinae and Epilachninae. He treated the subfamily Tetrabranchinae (=Lithophilinae) as a tribe lithophilinii and placed the group under Coccidulinae. Sasaji's classification was based on the extensive comparative morphology of both adult and larvae.) The above mentioned taxonomy is based on the traditional alphataxonomy, i.e, based on morphology. No work on the cytogenetics and taxonomy of the ladybird beetles have done in Bangladesh.

Cytogenetics an integrated discipline concerning the study of segregation of heritable variation aleing with the multifaceted behavioral pattern of chromosomes during cell cycle. The principle of establishment of cytogenetics was laid down with the universal recognisation of chromosomes as a super molecular system and cytogeneticists convincingly demonstrated that the chromosomes are the vehicles of hereditary materials of an organism from virus to mammals, where each and every genetical traits is ordered into one or more chromosomes (Stebbins, 1950). Thus chromosome was established as a matter of principle, a complex and highly ordered organelles, rather than a random array of genetic units (Benzer, 1955).

The study encompasses the 'Chromosome Science' in its fundamentals and advancements alongside its implications in various attributes of differentiation and evolution (Capanna *et. al.* 1977; Britton-davidian [illegible] 1990). Chromosome science with its great potential of future achievements has become completely a new synthetic science due to advancement of technical aspects of bio-chemistry, bio-physics, cell physiology and genetics (Sharma, 1984) Great interest accompanying the continued refinement in methodology gave an impetus to our understanding of the structure of chromosome from purely a cytogenetical level towards a cytochemical and cytophysical analysis (Sharma, 1976). It was a period of most rapid coherence of knowledge that biology has ever witnessed.

The universal occurence of chromosome indicates that there is a clear evolutionary sequence in the complexity of the chromosomes from bacteria to the higher organism (Sharma and Sharma, 1965).

The chromosome study has, therefore, attained an utmost importance for cytogenetical determinant and in establishing interrelatedness as well as phylogenetic pathways between the species (Sharma, 1960, White 1973, Vosa 1977). Any change more or less pronounced in the specific pattern of each genome must have some phylogenetic implication and should go a long way in speciation (Patton and Sherwood, 1982).

Chromosomal differences reflect in general diferences in the genic contents of the individuals. The major variations which can be observed from a comparison of related species can be divided into: variation in absolute chromosome size; variation in basic number; variation in number and position of satellites and variation in the degree and distribution of heterochromatic regions. (Sharma, 1976).

The variations in size may reflect either differences in the number of gene products or proteins produced by the individual or duplication of genes which influence the rate of synthesis of individual Proteins. Variation in chromosome morphology show alternation in gene arrangement. Alternations in chromosome number involve either differences in gene arrangement or gene duplication or deficiency or both. Chromosomal differences thus reflect differences in the source or gene variation while morphological, physiological and biochemical differences indicate variation in the products of gene action as modified by environment factor (Stebbins 1966).

The chromosomes in different organisms as well as in the same cell besides their absolute and relative sizes may show a definite individuality in their genomic pattern as are evident from their size, shape, position of centromere and in such additional features as secondary constriction and satellites (Stebbins, 1950). The Russian School of cytologist headed by S. Navaschin, developed the fundamentals of the karyotype concept from their observations that most species of living organisms show a distinct and constant individuality of their somatic chromosomes and that closely related species usually have similar chromosome complements and which in distantly related ones are often recognizably different (Navaschin, 1912).

Lewitsky (1924, 1931) referred the term karyotype as the phenotypic appearance of the somatic chromosome in contrast to their genic contents the term 'Idiogram' (Navaschin, 1912) is applied to the diagrammatic representation of the karyotype, which his son Navaschin Jr, (1925) referred for specific picture of chromosome of a species without citing any references. A detailed information as to the distinguishing characters by which karyotypes differ from each other was furnished in the classical works of Heitz (1928, 1932), Darlington (1937) and others like Baker *et al.* (1983); Hsu (1973), Gill *et al.*(1988) and Van Dyk (1990). The following aspects may be analyzed from the above basic number of chromosomes, form and relative size of the satellites and secondary constrictions, absolute size of the chromosome and staining properties, viz. euchromatin and heterochromatin (Stebbins, 1964).

The chromosome number is an important datum for a species than any other characteristics seemed significantly stable to merit taxonomic significance (Garber, 1978). Thus entire morphological, physiological and biochemical aspects are being altered (Sharma and Sharma 1965; Brinkly and Hittle man 1975; Brown, 1984).
In different organisms, chromosome is in the centric position of the cytogenetics. Flerencio & Saidul(1990) studied the mitotic salivary gland chromosomes of *Dacus dorsalis* in the cytogenetical analysis.

Like other animals many species of ladybird beetles show considerable intra specific variation in the marking of elytra, as well as the head and pronotum. These variations were taken by earlier taxonomists (kapur, 1940-73); Miyatake (1959-'80); Ghani (1962) as sufficient ground for subdividing a species into varieties or forms and for giving them different names. Occasionally individuals of the same species having distinct markings had been assigned to different species or even to different genera (Majerus & Kearns 1989). The marking of variable color patterns are composed dark design or a light (brownish, yellowish, reddish or whitish) back ground. The dark pigment is melanin, the light pigments have been found of contain derivatives of carotenoids. The carotenes are partly acquired in the food, partly synthesized de novo by the coccinellid, probably in co-operation with symbolic carotenogenic micro-organisms (Britton *et al.*1977).

A convenient way of classifying the variation depending on the elytral spot patterns, may be really complicated in some species. The beetles are classified according to the number of spots and fusions which roughly characterize the degree of melanisation (Mader, 1926-' 37).

Melanin is a group of polymers derived from the amino acids. Certain invertebrates and micro- organisms produce melanin pigments. Black colours of the body caused by the over production of the pigment melanin, often as a reaction of the environment. Epidermal pigmentation of the invertebrates are caused by different amino acids (Komai, 1956).

The heredity of color patterns has been studied by the genetic methods. The polymorphism is usually based on a series of multiple alleles, although perhaps there also exist examples of non allelic inheritance. Examples of earlier genetic studies of polymorphism in several coccinellids were reviewed by Komai (1956). Komai concluded that such series of multiple alleles determine the main color types of elytra and ensured that the stable and distinct polymorphism within each species is maintained. The minor peculiarities of pattern. Which undergo continual change are controlled by polygenes (modifers). Ford (1964) considered it more likely that such a multiple allele effects, as has been demonstrated in coccicellids which is caused by a sufficiently close juxtaposition of the loci of the genes, so that crossing over is most unlikely to separate them. Ford designates such a complex of loci as a "super gene".

Not only the coloration and the number of spots and pattern of their distribution and pattern of their distribution on elytra, but also a minor variation in the position of different spots, their size and shape are under genetic control. This type of variation was extensively analyzed in *Epilachna sp* by a group of German and Russian Authors working in Berlin in 1930's and reviewed by Timofeeff-Ressovsky (1940). These authors worked with five geographical sub-species of two different species, *Epilachna chrysomelina and E. capensis.* All subspecies have an identical pattern of six elytral spots differ in a number of small characteristics including spot size, shape and position on the elytral surface. The sturation of pigmentation of ground colour of the elytra, pronotum and ventral side and the extent of nonpigmented areas which may appear around the elytral spots are also variable. The overal quantity of elytral spot pigment is controlled polygenically (Komai 1956). Individual with all possible homo and heterozygote combinations of alleles occur in nature (Komai 1956). Variation in the color and patterns on the pronotum and elytras of the genus *Adalia* results in the speciation (Majerus *et al.*, 1987). It has recently been suggested that several species of ladybird employ both camoflage and warning coloration as defensive devices (Majerus, 1985).

It has also been suggested that many of the warning colored species of ladybirds are Mullerian mimics (Muggleton, 1978). In phytophagous lady beetles *Epilachna vigintioctomaculata* complex different host plants may also reflect on genetic variation which causes speciation (Katakura, 1976, 1981,1982, 1988, 1989, 2001).

All allelic series encoding the information that DNA contains does not immediately affect the cell only. When it is used to direct the synthesis is it able to determine a cell chemical-physical properties which would depend on the constituent amino acids.

All the genetic information of any individual are written in articulated language or coded language of nucleotides or nitrogen bases of DNA which can be translated into the amino acids sequences (Verma & Agarwal 1981).

The different combinations of amino acid give rise to large variety of proteins, determining their biological activity (Sharma 1976).

In some cases pigmentation can also depend on the composition of the medium and the temperature and also on the peptide chain of amino acids (Hans 1986). So, the amino acid may be a datum for a species.

Proper identification of different intra and inter specific varieties of ladybird beetles is needed to study their pest control activities. The study of Karyotype as well as the cytogenetics may be a precise approach for identification of suitable variety for biological method of pest control.

Because of their worldwide distribution and abundance, the ladybird beetles constitute a unique position in the cytogenetical literatures. According to Kitzmiller (1976), a substantial amount of cytogenetical work has been done in different parts of the world but there has been practically no such report in Bangladesh.

The present investigation incorporates the nature of chromosomal individuality as has been revealed in the concept of karyotype by analysing the karyomorphological pattern as well as the biochemical estimation through the qualitative analysis of amino acids among some ladybird beetles of Bangladesh. It has illuminated the genetical analysis in establishing the phylogenetic relationship among the species.

Amino acids are the alphabets of protein structure and determine many of the important properties of proteins (Lehininger, 1972). The importace of aminoacid in nature has been recognized ever scince the beginning of the twentieth century. It has a great role in gene sequence (Benzer, 1962).

There are only twenty amino acids that occur in proteins and these are found in all organisms. This implies that organism have evolved from a common ancestor that had also the essential amino acids (Thompson *et al.*, 1969). The surface of most coccinellids particularly the elytra with characteristic color patterns are due to some chemical and physical properties which would depend on the constituent amino acids (Sharma, 1976). The different amino acids in various proportions are attached by peptide bonds. The different combinations of these amino acids give rise to a large variety of proteins, determining the biological activities (Sharma, 1976). Thus the amino acids are also important causing for genetic variations. Amino acids occur in the cell as free amino acids as the polypeptide forms. Arrangement of amino acids in a polypeptides chain is the primary structure of protein. The peptide chain are made by hydrogen bond which can be broken by heat, pressure, pH, electricity heavy metals and other agents. On hydrolysis they yield only amino acids. Each amino acid has an amino group and carboxyl group. Free amino acids in the cell are linked to form polypeptide chains by the combinations of acetic group of one amino acid.

Hubby and Throckmorton (1967) discussed factor influencing the extent of genetic divergence at the protein level among species. Genetic differentiation during speciation have been discussed by Ayala *et al.* (1974). Sasaji and Ohnishi , 1973a studied the esterase isozyme in ladybird beetles , obtained some results from several species of the Coccinellidae.

On the other hand, sibling species which do not interbreed but are difficult to separate on morphological ground alone, have been studied, using biochemical analysis (Sharma,1976). *Harmoia yeonsis*, a sibling species of *Harmoia axyridis* was recognized for comparative studies on the many issue of this complex were carried from the view point of the biosystematics , for example reproductive isolation , gene frequency in natural population, amino acids sequences in protein structure , larval and adult morphological characters , life history, and karyotypes (Sasaji, 1981).

With the advent of techniques , study of amino acids received new dimensions regardless of source, the amino acids are normally identified first by paper chromatography , being recognized by an unusual position of color reaction with ninhydrin or the common spray reagents. Subsequent discoveries of thin layer chromatography (TLC), ion-exchange chromatography ,electrophoratic techniques, ultra violet and infra-red spectroscopy, gas chromatography and many other techniques have made the study of amino acids much easier (Chowdhury, 1970).

The present investigation has have not aimed at to provide comprehensive treatments of each area of chromosome dynamics and amino acid compounds, but by illustrating some basic concepts, questions and approaches attempt has been made in humble way to furnish a tentative working hypothesis in coccinelids cytogenetics, a field suffering from a serious lack of professional responsiveness in our country.

CHAPTER --- 2

REVIEW OF LITERATURE

Review of Literature

Works on faunistics, bionomics and cytogeneticists of the Coccinellidae in the world by various authors are numerous and reports dealing with those are scattered. Various Coccinellists like Fabricius (1798) and Mulsant (1846, 1850, 1853).

Mulsant (1850) reported several ladybird beetles *e.g; Coeloptera pupillata* (Swrtz), *Rodalia fumida* Mulsant, *Rodalia ruficulus* Mulsant, *Calvia dorsonata* Mulsant and *Scymnus nubilus* Mulsant from Bengal.

Crotch (1874) and others made classical contribution on the Coccinellidae from different regions of the world. However, an endeavor has been made to review literature for convenience, a comprehensive record of beneficial and harmful coccinellids in favor of cytogenetical studies of some coccinellids species . However, an endeavor has been made to present here a review of the literature for convenience, a comprehensive record of beneficial as well as harmful coccinellids in favor of the study of cytogenetics of the Coccinellidae.

A cursory glance at the literature may enumerate the high lights as follows: Strasburger's description of the densely stained bodies in the nucleus in 1875; Flemmings monitoring of the splitting of chromosomes when he coined the term chromatin in 1879 for stainable materials of the nucleus. Constancy of the chromosome number was determined by Weismann (1888) and Bovery (1893).
Gorham (1894 b) reported six species, namely *Adalia indica* (M. andrewes's coll), *Coccinella septempunctata* Linn. var. *divaricata*, *Oenopia sauzet* Muls *Cheilomenes sexmaculata* (= *Coccinella-6-maculata*) Fab.

In addition to Mulsant's report Weise (1895) added some other species including Epilachninae. He also reported some predator species namely *Chilocorus circumdatus* (Schonherr). *Synia melanaria* Mulsant. and some beneficial species viz.; *Adalia telaspilota* (Hope) , *Coccinella-7-punctata* Linn. , *Coccinella-9-punctata* Hbst. , *Chilocorus nigritus* Fab, *Ceilomenes sexmaculata* Fab.Casey (1899) also made classical contribution on the Coccinellidae.

Stevens initiated chromosome studies of this family as early as 1906, only 55 species have yet been explored cytologically (Bose 1948) and others.

Amino acid contents in the haemolymph of *Bombyx mori* L has been isolated by Yoshitake and Aruga (1950), Ishimori and Muto (1951) and Drilhon *et al.*, (1951,1952).

Again the paper reviewed for cytological reports on the Coccinellidae published by Smith (1953). In the same period with the advancement in chromosome methodology, it was reported by Hsu and pomerate (1953) that cell swelling and inhibitions of spindle formation were accompanied by hypotonic salt solution.

Later the reports were in hand on the amino acids contents in the salivary gland of some insects by Nuoroteva (1955, 1956). Nearer to this period works on coccinellids chromosomes were reported by Agarwal (1960,1961) and Smith (1960b, 1962).

While making a survey of the ladybird fauna of the paddy field in the orient, Sassaji (1968 b) recorded 33 species of which five species were from East Pakistan. In fac, he also described one new species viz. *Micraspis yasumatsui*. Sassaji in addition to *Brumoides lineatus* (Weise) and *Micraspid vincta* (Gorham) as new records from the territory.

For the chromosomal studies, the use of drugs like colchicine to separate the chromatids of prophase and metaphase chromosomes since the time of Pioneering works. Colchicine inhibits mitosis by causing disorganization of spindle formation.

However, the length of application of colchicine should be limited because long application sometimes may cause polyploidy (Prist, 1969).

Chromosomes of some coleoptera were studied by Dasgupta (1972), Dasgupta and Chakrabarti (1973), Kacker (1973) and Saha (1973). They studied on the chromosomal numbers and karyotypes of different species of sub-family, Coccinellinae and Epilachninae of the family Coccinellidae.

Further more, in favor of amino acids separation , more papers were reviewed on the enzyme polymorphism of coccieillidae by sasaji and Ohnishi (1973a, 1973b), Sasaji (1974), Tanimoto (1975).

Further, "The chromosomes" by Sharma (1976) has given informations on chromosomes in different point of view. Protein and polypeptides consist of the association in various proportions of some 20 different amino acids attached by peptide bonds also have suggested there.

A vast study on the phytophagous lady birds *Henosepilachna vigintioc tomaculata* complex was accomplished by katakura (1976) .

Again Sasaji and Hisano (1977), Kuboki (1978) and Sasaji (1981) showed the esterase polymorphism and their inheritance in *Harmonia axyridis*.

Coccinellids have been very actively studies on the course of the twenty three years since "Biology of Coccinellidae" was published The great amount of new, and other very important findings have made the previous book outdated and a new synthesis is needed. No other monograph of similar focus and extent has appeared Iablokoff-Khnozorian's "Les Coccinelles" (1982) limited to the tribes Coccinellinii, and Gordon's "The Coccinellidae of Hmirica North of Mexico" (1983) both concentrate on taxonomy. Here also reviewed a check list of Indian Coccinellidae with recorded host plants which was published by Anand *et al.* (1988).

Chromosome number and sex determining mechanism of some Indian Coleoptera was studied by Yadav & Dange (1989). Karyological investigations on some Indian Coccinellids was performed by O.P. Mittal *et. al.*, (1989).

Booth and Pope (1989) listed *Rodalia breviuscula* Muls., *Rodalia famida* Muls, *Rodalia sexnotata* (muls.), *Scymnus coccivora* Ayyar (= *Scymnus andrewsi*) , *Coccivora* Ramkr. *Sumnius nubilus* and *Micraspis discolor* (Fab). . Majerus & Kearns"ladybirds" (1989) deal specifically with British Coccinellids.

Tsurusaki *et.al.* (1993), studied the cytogenetics of a big part of the species of the *Epilachna vigintioctomaculata* complex under the subfamily Epilachninae
Rahaman reviewed the status of coccinellids research in Bangladesh upto 1995. He gave an uptodate historical reviewed of on the topic. He listed 52 species of ladybirds excluding phytophagous one. Professor Katakura identified four Epilachna beetles from Bangladesh. So far no more Epilachna has been found in Bangladesh.

A details information of coccinellid gained from the study of Ecology of Coccinellidea (Hodek & Honek,1999) Recently, the two spot lady beetle *Adalia bipunctata* has a great research value to the latest scientists (Hemiptinne *et al.*2000, 2001; Yasuda *et al.*2002; Magro *et al.* 2002).

As far the present investigation is concerned, although the lady beetles are well serve with reviews, the cytogenetical study is not very plentiful. Moreover reports on amino acids in coleoptera is wanting in the present investigation .
The pertinent literature of amino acid detection in Coccinellidae or even in coleoptera were scattered or shortened in the present studies.

In the backdrops of this historical perspective of the Coccinillids cytogenetics worldwide, the present investigation examined the karyo-systematic aspects of these various beneficial or harmful ladybird beetles exerting a massive impact in our agro-based economy by compatible pest management, alongside providing a great boon to the cytogenetical literatures attributed to the advancement of chromosome science. Besides describing the karyomorphometrical analysis, moreover chromatographical assessment on the amino acids contents of the species in the context have also the important cytogenetical information. Considering the great deal relevance the present studies has been initiated.

CHAPTER - 3

MATERIALS AND METHODS

Materials and Methods

The present assay for chromosomal delineation in metaphase utilized hypotonic salaine for cell exapansions as devised by Hsu and Patton (1969) combined with colchicine pretreatment and Giemsa / Orcein staining. All the operations were performed *in vivo* in the laboratory of the Institute of Biological Sciences, Rajshahi University. The protocols have been applied to cells obtained from gonodial cells of several ladybeetles of 20 species belonging to 16 genera.

Collection of Beetles

The beetles constituted for the present cytological investigation comprised of wild coccinellids belonging to the Family Coccinellidae of the Order Coleoptera. The beetles were collected from different ecological habitat and niches of Bangladesh. The most suitable time for collection of ladybird beetles was from early winter to mid summer, but the collecting efforts were made throughout the year. Specimens were collected by established methods; eye searching, hand picking, sweeping or beating vegetation in cultivated areas, arborata virgin orchards and new forests (Gordon, 1980 and Majerus and Kearns, 1989). Some specimens were collected in light traps. Occasionally lady birds from the upper canopy of trees collected using locally made ladder.

Identification and measurements of the samples of coccinellids beetles

Specimens were identified following the keys of Weise (1892,1895), Gorham (1894b), Ayyar (1925), Korschefsky (1930, 1933), Chapin (1940, 1962, 1965a, 1965b), Kapur (1942, 1943, 1946, 1948a, 1948b, 1949, 1950, 1951a, 1951b, 1956a, 1956b, 1958, 1961a, 1961b, 1963, 1966, 1969), Timberlake (1943), Diek (1947) Kamiya (1959, 1960, 1961a, 1961b); Miyatake (1961a, 1961b, 1970 1980), Sasaji (1967, 1968a, 1968b, 1971, 1994); Bielawsky (1972, 1979), Hodek (1973), Chapin (1974), Katakura (1981) Ioblokoff- khnzorian (1982), Gordon & Chapin (1983), Pope (1988), Booth *et. al.* (1990), Rahman (1991), Rahman and Ali (1992). Standard taxonomic measurements were taken in mm. scale.

The table 1 shows an overview account of the coccinellids species brought to the laboratory for the present cytogenetic survey.

Table 1 morpho-taxonomical account of some species of lady bird Beetles of the Family Coccinellidae used in present investigation

Species (sub-species)	Taxonomic status and habitat	Brief description
Rodalia fulvescens Hoang	Sub fam: Coccidullinae Beneficial insects commonly found on *Muraya paniculata* and *Bambusa sp.*	Hemispherical body 2.5-3.4 mm in length and 2.25-3.2 mm in width. Eyes large, convex, black, finely faceted and pubescent. Antenae eight segmented. Body surface entirely testaceous, also concolorous, pronotum broad and elytral epipleura entire.
Stethorus tetranychi Kapur	Sub fam: Scymninae Tribe: Stethorini Beneficial entomophagous insects very common in collection; like on *Acacia sp*	Small medium size body ranges from 1.2-1.35 mm in length and 0.85-0.9 mm in width. Black body shortly oval, broadst in the middle, convex moderately,elytral epipleura present.
Scymnus nubilus Mulsant	Sub fam: Scymninae Tribe : Scymini Beneficial insects found in *Mangifera indica, Morus sp. etc*	Oval body, moderate in size, generally1.5-2.2 mm in length and 1.2-1.4 mm in width. Dorsal surface reddish-brown to yellowish-brown. The middle of basal area on pronotum with a black clear marking. The black marking covering the sutural area of elytra. Convergent posteiorly.
Brumoides lineatus (Weise)	Sub fam: Chilocorinae Tribe: Platynaspini Benificial beetles, abundant in the plant *Morus spp., Solunum melongena* etc.	Body 3.4 to 4.25 mm in length and 2.0 to 2.5 mm in width , oval body glabrous. Head brown with prominent black eyes. Elytra yellowish-brown except at their basal, lateral and apical margines. Three black villae-a median extending from base to apex of elytra, two lateral extending from base of elytra to elytra tip. Antennae nine segmented, brown, bearing small sensory hairs.
Jauravia pullidula Motschulsky	Sub fam: Sticholotidinae Tribe: Sticholotidini World wide distribution.	Body broadly oval, small and brown, bearing fine deep and close punctations. Elytra with dense pubiscence. 2.2-2.4 mm in length and 1.8-2.00 mm in width.
Pharoscymnus taoi Sasaji	Sub fam: Sticholotidinae Beneficial entomophagous beetles, mostly found in the plants of *justicia gendarussa* L *Acacia sp, Morus sp.* etc.	Small short oval body 1.65-2.00 mm in length and 1.28-1.5 mm in width. Dorsum relatively strongly convex and pubescent. Head black Pronotum and scutellum entirely black. Elytra black with two pairs of reddish orrange marking before and behind. Underside of body dark reddish brown pitchy brown. Antennae shorter and ten segmented, mouth parts dark brown.

cont.

Illeis indica Timberlake	Sub fam:. Coccinellinae Tribe : Psylloborini Beneficial predators found in all collecting localities.	Body relatively large, 4.45- 5.5 mm in length and 3.6 – 4.00 mm in width, ground colour yellowish, less convex and distinctly narrowed apical region, pronotum narrowed anteriorly and widest at posterior angle and almost white with a pair of brownish black spots at its base.
Psyllobora bisoctonotata (Mulsant)	Sub fam: Coccinellinae Tribe: Psylloborini Beneficial beetles, generally aphidophagous.	Upper surface of the body glabrous and shiny yellow or pale yellow. Pronotal disc brown and elytral suture light brown, elytral margine in the configuration of 2-3-2-1; head and pronotum light brown, antenna 11 segmented. Body generally 2.6 – 3.2 mm in length and 2.00-2.35 mm in width.
Cheilomenes sexmaculata (Fabricius)	Sub fam: Coccinellinae Tribe. Coccinellini Beneficial, aphidophagous.	Short oval body, 3.8-5.5 mm. length 3.2 – 4.6 mm. width. Some are entirely black and some are brownish with parallel black stripes on elytrae.
Coccinella septempunctata Linnaeus	Sub fam: Coccinellinae Tribe: Coccinellini. Beneficial beetles found in cultivated vegetable areas.	Oval body with 3.5-5.25mm.in length and 3.0 – 4.0mm in width. Brownish elytra with 7 black spots in the configuration of 1-2-2-4; each spot nearly round, pronutum black with yellowish marking. Head and ventral surface black.
Coccinella transversalis Fabricius	Sub fam:Coccinellinae Tribe.:Coccinellini Beneficial beetles generally aphid feeders, found in cultivated areas.	Body rather rounded and convex. Length 6.5-7.5mm. and width 5.0-6.0mm. Red elytra with triangular black marking. Pronotum and head black. Ventral surface also black.
Harmonia octomaculata (Fabricus)	Sub fam.; Coccinellinae World wide distributed entomophagous beneficial beetles mostly found in rice field before ripening.	Body 4.5 to 6.25 mm in length and 4.0 to 5.0 mm in width. Body relatively less rounded and convex; dorsum reddish orange or reddish yellow with or without black marking. Pronotum black in the middle or with one or two pairs of black spots; elytron normally with a pair each of black, humeral, median and post median spots and a sub- apical spot. Suture also usually black.
Micraspis disclor (Fabricius)	Sub fam.: Coccinellinae Tribe. Coccinellini. Beneficial beetles abundant in various crops fields.	Hemispherical, glabrous body, 3.6 – 4.4 mm in length and 2.8 – 3. 49 mm in width. Orange-brown elytra with narrow black line along the suture. Pronotum and head black. Under surface, dark brown.
Micraspis yasumatsui Sasaji	Sub fam.: Coccinellinae Tribe. Coccinellini. Beneficial, abundant in crops fields.	Nearly hemispherical body with 4.00-5.00mm. length and 3.45-3.5mm. in width. Orange elytra without any dark area or black suture. Head and pronotum yellowish brown. Ventral surface entirely brownish

cont.

Propyeia quatuordecimpunctata Linneaus	Sub fam.: Coccinellinae Tribe :Coccinellini Beneficial insects feed on various sp. of aphis, found in the plant of *Solunum melongela, Brassica juncea* etc.	Oval body 2.25-4.3 mm in length and 1.75-3.5 mm in width.Weakly convex,glabrous, upper surface brownish, pronotum with a large black spot except its lateral margine.Angulate anterior corners of pronutum. Elytral base broader than pronutum.
**Apomycraspis quayumi* Ali & Rahaman (nearer to *Micraspis)*	Sub fam: Coccinellinae Tribe: Coccinellini Beneficial entomophagous beetles found in *Bambusa sp.* etc.	Body elongated sub-oval or hemispherical,glabrous,convex, less shining, dorsal surface tastaceous, eyes black, head width, antenna half as wide as head, maxillary pulp four segmented, pronotum wide. Elytra very slightly convex above. 2.7 to 2.9 mm in length and 2.5 to 2.55 mm in width.
Afidenta misera Mulsant	Sub fam: Epilachninae Type - Epilachnini Phytophagous harmful beetles mainly pest on bean.	Smaller in size, grayish-brown in colour. Body length varies from 5.2-6.00 mm and width 4.9-5.5 mm. Pronotal spots generally absent, each elytron with 6 spots.
Epilachna septima Dieke	Sub fam: Epilachninae Type - Epilachnini Harmful phytophagous beetles, serious pest of bitter-gourd. Both the larvae and adults feed on the leaves and generally completely defoliate.	Body spherical in shape. generally 4.00-7.5 mm in length and 2.5-5.5 mm in width. The external median black spots of the elytra generally not touching the external margin of elytron.
Epilachna pusillanima Mulsant	Sub fam: Epilachninae Type - Epilachnini Phytophagous harmful beetles generally pest on cucumber.	Moderate in size; length varies from 4.5- 7.5 mm and width from 3.00-5.5 mm. Elytral apex rounded, brownish in colour with black spots. The external, median black spot of the elytra generally touching the external margin of elytron; male genetalia with the median lobe dentulate dorsally.
Epilachna vigintioctopunctata Fabricius	Sub fam: Epilachninae Tribe : Epilachnini Harmful phytophagous beetles abundant in vegetarian fields, arborata and also in other crops fields.	Spherical shaped insects 5.00-8.00 mm in length and 3.00-6.00 mm in width. Elytra brown with 12-24 black spots. Pronotum also brown. Ventral surface light brown.

- Only the proposed name, taxonomically unpublished

Laboratory procedures involving testicular tissue preparation of coccinellids

Almost similar general laboratory techniques were employed for all the species of ladybird beetles.

Sex distinction

It was difficult to distinguish between male and female ladybird beetles. In most species the females were slightly larger than the males and there were small differences in shape but those criteria were not totally reliable. Careful examination of the inter targal connective of the abdomen of some species of ladybirds revealed sexual differences in most case, those were best seen with a low power dissecting microscope. Surprisingly there was no single set of criterion for sexing all the species, as each has its own distinctive feature. In some species the abdomen was pointed in the female, in other in the male. In some cases the cuticular plates were notched in the male, or undulating in the female. The only feature found in all males and absent in all females that had examined, was three curved bands of thin flexible dorsal cuticle at the abdominal segments were leathery and broader in male (Majereus and Kearns 1989).Only the male beetles were selected for the present investigation and the testicular follicles were collected through the dissection of male beetles.

Dissection.

Male reproductive systems were dissected out by removing the abdominal tergites and testiculat follicles were collected for chromosomal preparations.

Assays for the meiotic metaphase chromosomes preparetion for testicular cell of coccinellids.

The preparation of cellular materials for chromosome analysis of the coccinellids consists of the following basic methods as has been indicated earlier. Almost similar general laboratory protocols were applied for all the species excepting few minor modifications as and when required in some stages of the procedure. An effort was also made to assimilate various assays to correlate with good metaphase chromosome preparation.

A. Squash method (Darlington & laCour, 1976)

Testicular follicles were dissected out in Ringer solution (0.65 gm. NaCl + 0.25 gm. CaCl2 + 0.02 gm. NaHCO3 + 100 cc. distilled water). On a drop of 1% aceto-orcein (1gm. of orcein stain powder + 22 cc. glacial acetic acid + 4 cc. distilled water + 28 cc. lactic acid) or a drop of aceto carmine (0.5 gm. carmine powder + 45 cc. glacial acetic acid + 55 cc of distilled water) stain was taken on a slide, a follicle of beetle testis was kept for 10 minutes for staining. Some times a gentle heat was applied with the help of a spirit lamp. Then a cover- slip was placed on the stained tissue. By placing a piece of blotting paper on the coverslip, the follicle was squashed by pressing firmly with the help of a thumb. Then the follicular cells were dissociated. Slides were ready to study.

B. Air drying technique

The squash method sometimes were not up to the expectation. So an air- drying technique (Crozier, 1968) was adopted with some modifications (Mttal *et al.* 1989 & Tsurusaki *et al:* 1993). This technique consists the following steps:

i Pretreatment

Th selected beetles were pretreated with 1% colchicine solution (1gm. of colchicine powder in 100 ml. of distilled water). The beetles were punctured with a minuten pin and placed them in a small watch glass containing some colchicine solution for about 4 (four) hours at room temperature.

For the prevention of spindle formation, suppressing of cell division and chromosomal spreading in testicular cells, it was appropriate to treat with colchicine.

ii Hypotonic treatment

The pretreated beetles were dissected out in the hypotonic soluion of sodium citrate (1 gm. of sodium citrate powder in 100 ml. of distilled water) on a grooved slide and the desired organ, testicular follicles were transferred to fresh hypotonic solution on another grooved slide using a dissecting needle. The hypotonic solution was hanged in order to remove any debris. The total hypotonic treatment was allowed not exceeding 20 minutes and was carried out at room temperature.

iii Fixation

For increasing nuclear and chromosomal spreading, the selected cells were treated with 45% acetic acid in water, which was removed shortly and the material transferred to freshly prepared fixative; carnoy's fluid (3 parts of absolute methanol + 1 part of glacial acetic acid) and leave for 30 minutes at room temperature.

iv Chromosome spreading

For the rapid dissociation and good chromosome spreading, the tissue was transferred to a drop of 60% aqueous acetic acid on a warmed slide. Slight maceration was needed for proper dissociation.

v Slide preparation

Another drop of carnoy's fluid was added to the preparation and tilt the slide in all directions to ensure a maximum spreading. The slide was then warmed gently over a flame of spirit lamp, which assist dispersion and evaporation. The dried slides were placed in acetic ethanol (1 part of glacial acetic acid in 3 parts of absolute ethanol) for about 4 (four) hours to reduce cytoplasmic staining.

vi Chromosome staining

A number of chromosome stains were tried with varying degree of success like aceto-carmine, aceto-orcein, giemsa stain (1 gm. of giemsa powder + 60 cc. methyl alcohol + 60 cc. distilled water). Staining with 1% of aceto- orcein made good result. Staining procedure outlined by Imai (1966.) was followed to spread the stain. A coverslip was placed over the tissue and to spread chromosomes, the tissue was squashed with applying pressure on the coverslip by the thumb. Thus the slide became ready for chromosomal study.

vii Mounting

The stained slides were mounted carefully only with the coverslip, no mounting medium was used because the refractive index of canada balsam or DPX does not coincide with the chromosomes. So the tissue was mounted by attaching with the normal gum or nail polish only surrounding the coverslip.

The slides were prepared to study the chromosomes and micro-photographed under oil-immersion lens.

viii Karyotypes from micro photographs

Some of the microscopical slides were photomicrographed to provide some details for karyotypic analysis. For karyotyping and microphotometrical analysis metaphase was selected on the basis of their technical qualities from well spread metaphase plate examined with oil immersion magnification (x 1000). Earlier, a low power magnification was used to select spread metaphase and then oil immersion was used to photograph metaphase chromosome with a zeiss photomicroscope and a 100x oil immersion objective.

Photomicrography was meticulously standardized, having taken care as to the exposure time in zeiss photomicroscope.

The following steps were employed to make a karyotype of the chromosomes (Prist, 1969):

1. Photographs of each metaphase were taken. One photograph was cut apart and the other was kept intact for orientation and reference or to tally with original metaphase or for the use in case a cut out chromosome was misplaced.

2. The individual chromosome was cut out. In case of overlapping of the chromosomes, the whole configuration was removed together which was separated after the other chromosomes were taken apart.

3.The individual chromosome was glued down in decreasing order of size. That complete line up of the chromosome set constitute the karyotype.

4. In order to display the karyotype pictorially, the individual metaphase chromosome from the photograph were arranged by length and in pairs, later aligned in such a way that the centromeres were at the same level and the short arms were oriented upward. The karyotypes were prepared by taking photographs once again.

5. Centromeric formula was derived on the basis of l/s ratio proposed by Levan *et al.* (1964)

6. Morphometric analysis was done from direct measurement by ocular micrometer, the scale of which was earlier standardized with the stage micrometer.

Assay for the findings of amino acids composition of coccinellids species

Extraction was collected by the preparation of protein hydrolysate as adopted by Clark (1963), from the male members of 12 species of the family Coccinellidae .

In this experiment, the samples were hydrolyzed by treatment with both acid and alkali. Qualitative analysis of the constituents of the hydrolysates was carried out by one-dimensional thin-layer chromatography. In this section of the experiment, the neutralized extracts were chromatographed on thin-layer chromatographic (TLC) plate in a freshly prepared solvent system. The amino acids are located on the chromatograms by both the ninhydrin (ninhydrin spray) and iodine reactions (in iodine chamber).

The same materials and method was applied during the present investigation.

Acid hydrolysis

About one gm. of whole (a few whole male) coccinellid beetles of the same species was taken in a 50 ml. Erlenmeyer flask and 10 ml. of 8N H2SO4 was added. The top of the flask was plugged with cotton and was placed in autoclave under 15 lbs of. pressure for 5 hours. Then the sample was allowed to cool. The hydrolysate was neutralized by warming the solution with solid barium hydroxide. After each addition of barium hydroxide a time was allowed for proper dissolution and then the next portion was added. Neutrality of the solution was not allowed to exceeded. pH paper was used as a pH indicator . This process was continued up to that stage where the neutralization was attained to pH 3-4 or higher by adding saturated barium hydroxide solution. White precipitate was removed by filtration. The clear solution was taken for further analysis.

Alkaline hydrolysis

About another one gm. of the same tissue with 10 ml. of boiling water and 6.36 gm. of barium hydroxide; $Ba(OH)_2 \cdot 8H_2O$ was taken in another 50 ml. Erlenmeyer flask and in the same way top of the flask was plugged with cotton and was warmed gently while most of the $Ba(OH)_2$ was allowed to dissolve. Precautionary measures were adopted to avoid the formation of barium carbonate ($BaCO_3$). Then the flask was also placed in the autoclave at 15 lbs. with the previous one for 5 hours. After cooling the solution was titrated by adding about 2.5 ml. of 16N H_2SO_4 . The pH of the solution was checked with pH paper after adding acid at each step. When the pH was dropped to 10, less concentrated H_2SO_4 was used, until the expected pH (3-4) was reached. The solution was centrifuged and was washed twice with 5 ml. portions of boiling water. It was then made up to 25 ml.; with distilled water and hydrolysate was stored under low temperature for further analysis.

TLC (Thin layer chromatography) Plates

For all the runs TLC plates were prepared by spreading an aqueous slurry of finely ground solid of silica (10 gm. in 100 ml.) onto the clean surface of glass plate. The plate was then allowed to stand until the layer had set and adhered tightly to the surface. Then the plate was heated in an oven for several hours at 45° C. temperature.

Solvent system (Clark 1963)

The following solvent system was used for unidimensional TLC:

n-Butanol : acetic acid : water (12 : 3 : 5)

Colour reagent (Chowdhury 1970)

Ninhydrin ....0.2 % in acetone (w/v)

Iodine crystal......a layer in developing chamber.

Developing procedure for TLC (Clark 1963)

The TLC plate were developed unidimensionally to placing a drop of the sample near one edge of the plate by the side of known standard amino acids and marked its position with a pencil. After the sample solvent had evaporated, the plate was placed in the ascending way in a closed container saturated with vapors of the developing solvent, with care being taken to avoid direct contact between the sample and the developer. After the developer had traversed two-thirds of the plate, the plate was removed from the container and was dried. The position of the amino acids were determined placing the plate in an iodine chamber or spraying with ninhydrin which formed colored spots. The color developed in iodine chamber, was vanished immediately. Pencil lines were drawn around the individual spots.

The spots were identified by comparison of their position with those of standards which were calculated by Rf values.

Calculation of Rf values

Rf values of each spot was calculated by the following formula :

$$Rf = \frac{\text{Distance traveled by the solute (cm.)}}{\text{Distance traveled by the solvent (cm.)}}$$

Rf Values of the extracts' composition were compared to the Rf values of the standard amino acids.

Table 2 Summary of the Experimental procedure for Thin layer Chromatpraphy (TLC)

TLC Plates	Silica gel preparation / Ready plate
Solvent system: **One dimension**	n-Butanol: Acetic acid: Water (12: 3: 5)
Methods	Ascending technique in saturated chamber
Temperature	$(28\pm2)^0$ C
Distant	Was not constant
Detection	Iodine crystal & Ninhydrin soltion in acetone (100^0C)

CHAPTER - 4

RESULTS AND OBSERVATIONS

Results and observations

The various tables represent information at several levels of observations: chromosome numbers, types of chromosome, number of major arms, arms ratio, centromeric indices, relative length of chromosomes. The typical diploid complements of each of the 20 species as revealed by meiotic metaphase were displayed after karyotype of each species was prepared from well spread metaphase plates. On the basis of the karyomorphometric analysis respective tables were constructed and presented as and where required. In all 15 scoreable metaphase plates have been studied for each species revealing the normal diploid chromosome complements.

Other morphometrical data including centromeric positions, fundamental number, arm-ratio, relative length of chromosomes have been given species wise. Mentions were also made regarding the chromosome numbers with its karyomorphology (Table 3).

Chromosome number and morphology

The chromosome number and morphology promise to provide cytogenetic evaluation of the species under study. The constancy of chromosome number in all the species under investigation of 15 cells selected at random for each species, the chromosome count was fairly representative of all the normal cells. although the morphological and numerical changes were found in few instances, the result might be natural or due to handling effect or chemical treatment (Crozier,1968).

Rodalia fulvescens Hoang (Table 4, plate 1)

This lady beetle exhibited a consistent diploid number of 20. The karyotype analysis revealed that the autosomes were 5 pairs sm, 2 pairs metacentric and 2 pairs telocentric. The X chromosome was metacentric and largest while the y

Table 3 Gross cytogenetical results of the twenty species of coccinellids beetles

Name of the species	Diploid number of chromosomes (2n)	Karyomorphology Nos m	Nos sm	Nos t		Numbers of amino acids detected
Rodalia fulvescens Hoang	20	2,9,X ,	1,3,4,5,8	6,7	y	
Stethorus tetranychi Kapur	20	7,8,9 X	1,2,3,6	----	y	
Scymnus nubilus Mulsant	14	1,2,3,5,6 , X	4	--	y	
Brumoides lineatus (Weise)	18	8, X	1,4,5,7	2,3,6	y	
Jauravia pallidula Motschulsky	18	1 --- 5,X	6,7	8	y	
Pharoscymnus taoi Sasaji	20	2,5 -- 9,X	1,3,4	----	y	
Illeis indica Timberlake	20	X	1,2,3,4,9	5,6,7,8	y	10
Psyllobora bisoctonotata (Mulsant)	18	1,2,7,X	3—6 ,8	-----	y	09
Cheilomenes sexmaculata (Fabricius)	20	1 ----9, X	-------	--------	y	09
Coccinella septempunctata Linnaeus	20	7,--9	3,4,6,8, X	1,2,5	y	11
Coccinella transversalis Fabricius	20	1--- 9, X	------	-----	y	11
Harmonia octomaculata ((Fabricus)	16	1 ---7,	X	---	y	10
Micraspis disclor (Fabricius)	20	5	2,3,4,6,7,8,X	1,9,	y	10
Micraspis yasumatsui Sasaji		3 -- ,9	1,2, X	----	y	09
Propylia qu - atuodecimpunctata Linnaeus	20	1,5,9, X	2,3,6,7	---	y	
**Apomycraspis quayumi* Ali & Rahaman (nearer to *Micraspis)*	20	8,9,X	1,2,3,4	5,6,7	y	
Afidenta misera Mulsant	14	3,4,6	2,5, X	1	y	09
Epilachna septima Dieke	20	6 –9, X	1,2,4,5	---	y	08
Epilachna pusillanima Mulsant	16	5,6, X	1 ---4, 7	-----	y	10
Epilachna vigintiactopunctata Fabricius	18	1,2,3, 6,7,8,,X	4,5	-------	y	10

PLATE -1

Rodalia fulvescens Hoang

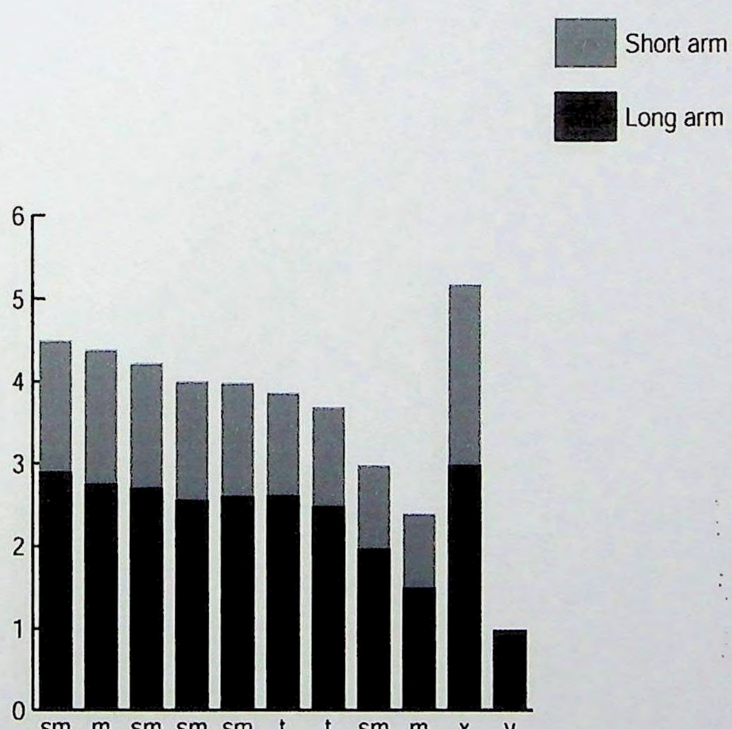
FIG- A

Metaphase chromosomes of *Rodalia fulvescens* Hoang

FIG- B

Karyotype of the metaphase chromosomes of *Rodalia fulvescens* Hoang

FIG- C



The Idiogram in spermatogonial cell of *Rodalia fulvescens* Hoang.

FIG- D

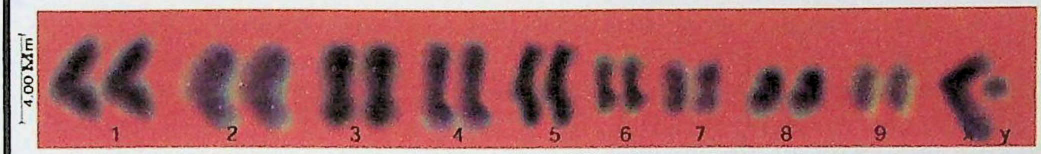
PLATE -2

Stethorus tetranychi Kapur

FIG- A

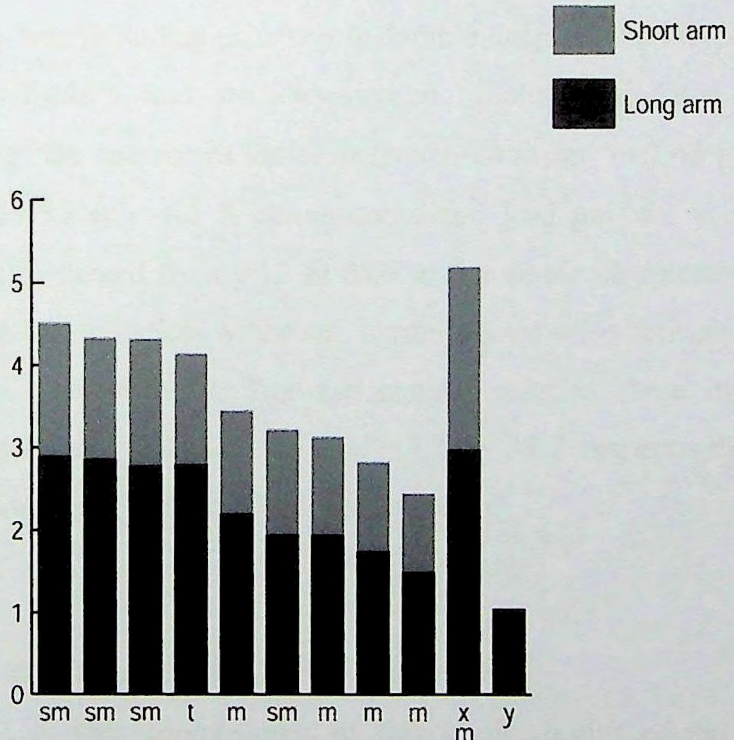
Metaphase chromosomes of *Stethorus tetranychi* Kapur

FIG- B



Karyotype of the metaphase chromosomes of *Stethorus tetranychi* Kapur

FIG- C



Showing the Idiogram in spermatogonial cell of *Stethorus tetranychi* Kapur

FIG- D

chromosome,was smallest of the complemement Centromeric position on ychrosome was indistinct. Of the AA the largest chromosome measured 4.5 μm while was 2.39μm (Table 4 and Fig a in Plate 1).The length of the X and the y the smallest chromosome were 5.17μ.m and 0.98μm respectively. The relative length showed a range between 0.13 to 0.06. Arm ratios of the sm chromosomes varied from 1.97 to 1.75 to that of the metacentric and the telocentric from 1.67 to 1.36 and 2.08 to 2.06. The centromeric indices ranged within 42.36 to 32.38.

Stethorus tetranychi Kapur (Table 5, plate 2)

The diploid chromosome number of this species is 20 as revealed by the majority of the chromosome plates. The karyotype was made by arranging the chromosomes in gradual series of length rather arbitrarily. The chromosome had the following break up : of the AA 4 pairs are sm , 4 pairs m, and a single pair t as well as the X chromosome was m and as usually y is dumble shaped. The morphometric data are appended in Table 5 and its karyotype in Plate 2. Tabulated data showed the total length of the autosomes varied between 4.45 μm to 2.45 μm while the sex chromosomes 5.2 μm for X chromosome and 1.05 μm for y chromosome. The relative length ranged from 0.12 to 0.03 in the whole complement. The arm ratio and the centromeric indices of the sm chromosomes were between 1.93 to 1.76 and 36.23 to 34.1 respectively. The metacentric pairs in those measurements had indicated between 1.65 to 1.34 and 42.7 to 37.7 respectively while those in telocentric ones were 2.07 and 32.5 respectively.

Scymnus nubilus Mulsant (Table 6, plate 3)

The analysis of the chromosomes of this small species of the ladybird beetles revealed a diploid number of 14 unlike the typical forms of the Coccinellidae. The karyotypic analysis exhibited the following break up : only a single pair was sub-metacentric and the rest were metacentric including X chromosome. y was as

Table 4 Morphometric data for the spermatogonial chromosomes of *Rodalia fulvescens* Hoang

Chrom pair	Mean length of short arm S± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenro meric type
1	1.60 ± 0.0064	2.90 ± 0.0075	4.50	0.1121	11.21	35.55	1.81	Sm
2	1.64 ± 0.0052	2.75 ± 0.0072	4.39	0.1094	10.94	37.36	1.67	m
3	1.52 ± 0.0153	2.70 ± 0.0064	4.22	0.1051	10.51	36.02	1.77	sm
4	1.45 ± 0.0066	2.55 ± 0.0167	4.00	0.1000	10.00	36.25	1.75	sm
5	1.38 ± 0.0072	2.60 ± 0.0062	3.98	0.0991	09.91	34.67	1.88	Sm
6	1.25 ± 0.0065	2.61 ± 0.0072	3.86	0.0962	09.62	32.38	2.08	t
7	1.20 ± 0.0156	2.48 ± 0.0171	3.68	0.0917	09.17	32.61	2.06	t
8	1.00 ± 0.0076	1.97 ± 0.0080	2.97	0.0740	07.40	33.67	1.97	sm
9	0.89 ± 0.0070	1.50 ± 0.0067	2.39	0.0595	05.95	37.24	1.68	t
X	2.19 ± 0.0143	2.98 ± 0.0071	5.17	0.1288	12.88	42.36	1.36	m
Y	0.98 ± 0.0052	————	0.98	0.0244	02.44	-----	-----	—

Total loength of the genome =40.14 µm: 2n =20: Karyotype= 5 AA sm + 2 AA m + 2 AA t + X m + y.

Table 5 Morphometric data for the spermatogonial chromosomes of *Sterthorus tetranychi* Kapur

Chrom pair	Mean length of short arm S ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.62 ± 0.0073	2.90 ± 0.0066	4.52	0.1169	11.69	35.40	1.79	sm
2	1.48 ± 0.0068	2.86 ± 0.0145	4.34	0.1122	11.22	34.10	1.03	sm
3	1.55 ± 0.0078	2.78 ± 0.0074	4.33	0.1120	11.20	35.80	1.79	sm
4	1.35 ± 0.0064	2.80 ± 0.0076	4.15	0.1073	10.73	32.50	2.07	t
5	1.25 ± 0.0134	2.20 ± 0.0082	3.45	0.0892	08.92	36.23	1.76	m
6	1.27 ± 0.0076	1.95 ± 0.0067	3.22	0.0833	08.33	39.44	1.53	sm
7	1.18 ± 0.0068	1.95 ± 0.0073	3.13	0.0894	08.94	37.70	1.65	m
8	1.08 ± 0.0154	1.75 ± 0.0064	2.83	0.0732	07.32	38.16	1.62	m
9	0.95 ± 0.0173	1.50 ± 0.0081	2.45	0.0634	06.34	38.78	1.58	m
X	2.22 ± 0.0058	2.98 ± 0.0071	5.20	0.1345	13.45	42.70	1.34	m
y	1.05 ± 0.0054	----------	1.05	0.0271	02.71	-------	----	—

Total loength of the genome = 38.67 µm: **2n** =20; Karyotype = 4 AA Sm +4 AA m + 1 AA t + X usually without distinct centromere. The mean total length of the largest chromosome of the complement measured 4.82μm which was the X chromosome chromosome was only 1.10μm (Table 6 & Plate 3). Mean total length of the genome was 25.61 μm. Relative length value ranged from 019 to 0.07 and the value of centromeric indices varied from 42.44 to 36.02.

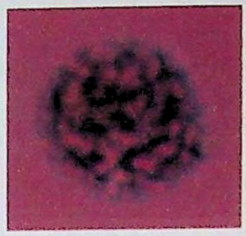
Brumoides lineatus **(Weise) (Table 7, plate 4)**

The diploid chromosome number asigned to this species is 18 as revealed by the majority of the chromosome plates. The karyotype was made by arranging the chromosomes in gradual series of length rather arbitrarily (Plate 4). The chromosomes had the following break up : 1-7 pairs of autosomes were sub-metacentric and the rest of the autosome and also the X chromosome were metacentric while the y was dumble shaped. The analysis was carried out of 15 metaphase plates from 5 individuals. The morphometric data are appended in table 7 which showed that the mean total length of the autosome varied between 5.29 μ m to 3.1 μm while the sex chromosomes measured to be 5.34 μm for X chromosome and 1.12 μm for the y chromosome. The relative length ranged from 0.13 to 0.07 in the whole complement. The arm ratio and the centromeric indices of the sub-metacentric chromosome pairs 1-7 were between 2.07 to 1.85 and 34.85 to 32.36 respectively. The metacentric chromosome pairs amongst the autosomes had relative length 0.07 whose arm ratio and centromeric index were 1.69 and 37.1 respectively. The relative length of the metacentric X chromosome was 0.13 and the centromeric index and arm ratio were 41.01 and 1.43 respectively while the y chromosome was also too smaller to detected the centromere.

PLATE -4

Brumoides lineatus (Weise)

FIG- A

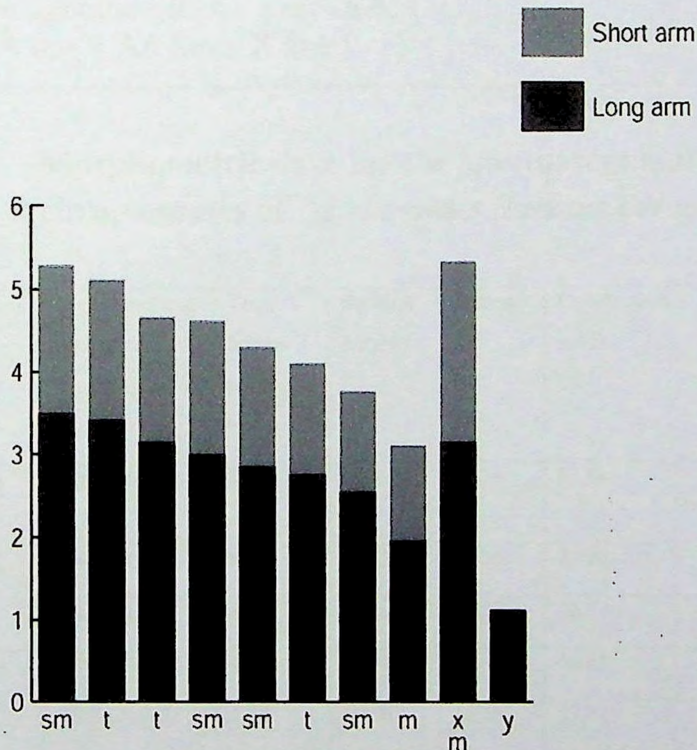


Metaphase chromosomes of *Brumoides lineatus* (Weise)

FIG- B

Karyotype of the metaphase chromosomes of *Brumoides lineatus* (Weise)

FIG- C



The Idiogram in spermatogonial cell of *Brumoides lineatus* (Weise)

FIG- D

Table 6 Morphometric data for the spermatogonial chromosomes of *Scymnus nubilus* Mulsant

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent . of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.64 ± 0.0065	2.44 ± 0.0064	4.08	0.1593	15.93	40.19	1.48	m
2	1.60 ± 0.0048	2.17 ± 0.0058	3.77	0.1472	14.72	42.44	1.36	m
3	1.42 ± 0.0055	2.15 ± 0.0732	3.57	0.1394	13.94	39.77	1.51	m
4	1.25 ± 0.0072	2.22 ± 0.0535	3.47	0.1355	13.55	36.02	1.78	sm
5	1.15 ± 0.0103	1.90 ± 0.0081	3.05	0.1191	11.91	37.70	1.65	m
6	1.10 ± 0.0075	1.65 ± 0.0066	2.75	0.0683	06.83	40.00	1.50	m
X	1.92 ±0.0054	2.90 ±0.0075	4.82	0.1882	18.82	39.83	1.51	m
Y	1.10 ± 0.0142	------------	1.10	0.0430	04.30	-------	-----	

Total loength of the genome = 25.61 µm: 2n =14
Karyotype = 5 AA m + 1 AA Sm + X m + y.

Table 7 Morphometric data for the spermatogonial chromosomes of *Brumoides lineatus* (Weise)

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	percent of RL RL%	Centromeric index CI	Arm ratio AR	Centromeric type
1	1.79 ± 0.0046	3.50 ± 0.0051	5.29	0.1276	12.76	33.84	1.95	Sm
2	1.70 ± 0.0042	3.42 ± 0.0073	5.12	0.1236	12.36	33.20	2.01	t
3	1.52 ± 0.0067	3.15 ± 0.0062	4.67	0.1127	11.27	32.54	2.07	t
4	1.62 ± 0.0048	3.00 ± 0.0060	4.62	0.1115	11.15	34.85	1.85	sm
5	1.45 ± 0.0053	2.85 ± 0.0055	4.30	0.1038	10.38	33.72	1.96	sm
6	1.35 ± 0.0041	2.76 ± 0.0054	4.11	0.0992	09.92	32.84	2.04	t
7	1.22 ± 0.0044	2.55 ± 0.0067	3.77	0.0998	09.98	32.36	1.88	sm
8	1.15 ± 0.0053	1.96 ± 0.0058	3.10	0.0748	07.48	37.10	1.69	m
X	2.19 ± 0.0084	3.15 ± 0.0070	5.34	0.1287	12.87	41.01	1.43	m
Y	1.12 ± 0.0045	------	1.12	0.0270	02.70	-------	----	

Total length of the genome = 41.44 µm. : 2n = 18.: Karyotype = 4 AA Sm + 3 AA t + 1 AA m + X m + y.

Jauravia pullidula Motchulsky(Table 8, plate 5)

A total number of 18 chromosomes including autosomes and sex chromosomes were found. Of the 8 pairs of AA first 5 pairs were metacentric, values of arm ratio of which ranged from 1.48 to 1.7. The value of arm ratio of 2 pairs of sub-metacentric chromosomes were 1.8 & 1.71 while the last single telocentric pair of AA showed the value of arm ratio 2.33. Of the sex chromosomes X chromosome was metacentric type whose value of arm ratio was 1.47 and the y chromosome was dot shaped without distinct centromere. Of the whole complement X was largest in length which measured 4.95 μm. Its relative length was 0.15 and centromeric index was 40.4. The mean total length of the AA varied from 4.19μm to 2.66 μm, the relative length and the centromeric indices of which ranged between 0.13 to 0.09 and 40.33 to 30. 04 respectively (Table 8 & plate 5).

Pharoscymnus taoi Sasaji (Table 9, plate 6)

In this small ladybird beetle , the diploid set showed the typical 2n=20, normal karyotypes of which were mostly metacentric (m), only 3 pairs of autosomes were sub-metacentric (sm) while arm ratio of y was unmeasurable. Of the sex chromosomes X measured 5.21 μm in lengthwhich was the largest. Mean total length of the whole complement and that of the relative length, centromeric index, arm ratio 0.14, 42.42 and 1.36 respectively. y measured 1.00μ.m in length. The mean total length of the AA ranged from 4.97 μm to 2.48μm as well as the ratio of relative length, centromeric indices of those ranged between 013 to 0.06 and 55.31 to 35.64 respectively (Table 9 and plate 6).Of the AA the arm ratios of the metacentric chromosomes varied from 1.56 to 1.18 and those of the sub-metacentric varied from 1.8 to 1.72.

PLATE -5

Jauravia pullidula Motchulsky

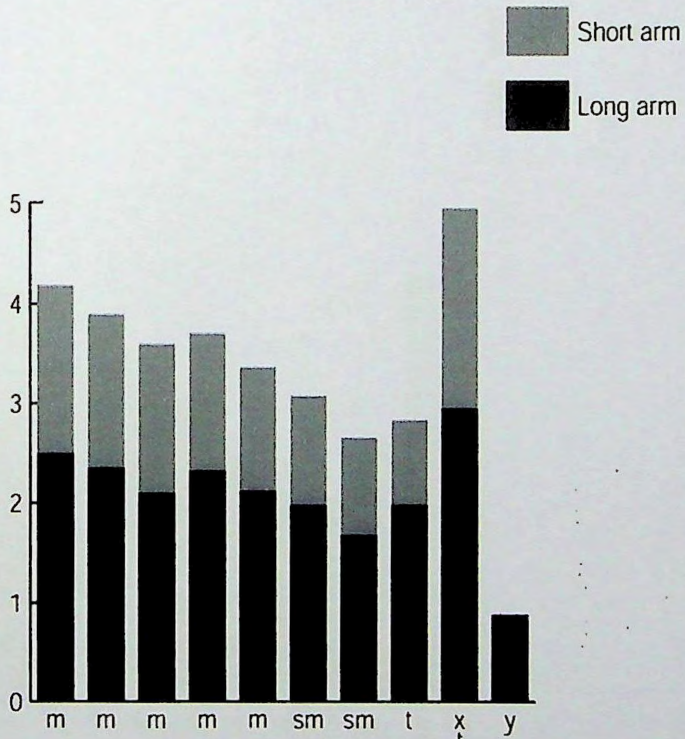
FIG- A

Metaphase chromosomes of *Jauravia pullidula* Motchulsky

FIG- B

Karyotype of the metaphase chromosomes of *Jauravia pullidula* Motchulsky

FIG- C



The Idiogram in spermatogonial cell of *Jauravia pullidula* Motchulsky

FIG- D

PLATE -6

Pharoscymnus taoi Sasaji.

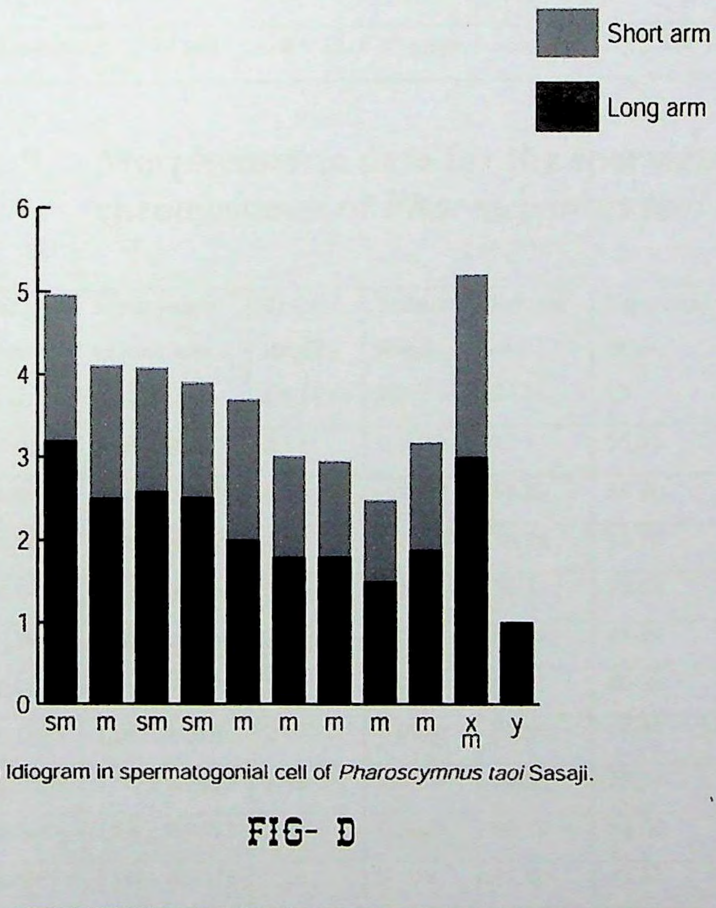
FIG- A

Metaphase chromosomes of *Pharoscymnus taoi* Sasaji.

FIG- B

Karyotype of the metaphase chromosomes of *Pharoscymnus taoi* Sasaji

FIG- C



Showing the Idiogram in spermatogonial cell of *Pharoscymnus taoi* Sasaji.

FIG- D

Table 8 Morphometric data for the spermatogonial chromosomes of *Jauravia pullidula* Motchulsky

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent . of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.69 ± 0.0075	2.50 ± 0.0095	4.19	0.1369	12.69	40.33	1.48	m
2	1.55 ± 0.0083	2.35 ± 0.0062	3.90	0.1176	11.76	39.74	1.52	m
3	1.50 ± 0.0146	2.10 ± 0.0058	3,6	0.1085	10.85	42.00	1.40	m
4	1.39 ± 0.0053	2.32 ± 0.0078	3.71	0.1118	11.18	37.50	1.67	m
5	1.25 ± 0.0124	2.12 ± 0.0142	3.37	0.1016	10.16	37.09	1.70	m
6	1.10 ± 0.0063	1.98 ± 0.0066	3.08	0.0929	09.29	35.71	1.80	sm
7	0.98 ± 0.0052	1.68 ± 0.1253	2.66	0.0802	08.02	36.84	1.71	sm
8	0.85 ±0.0124	1.98 ± 0.0077	2.83	0.0853	08.53	30.04	2.33	t
X	2.00 ± 0.0061	2.95 ± 0.0052	4.95	0.1492	14.92	40.40	1.47	m
y	0.88 ± 0.0071	---------	0.88	0.0265	02.65	-------	----	---

Total length of the genome = 33.17 µm . : 2n = 18. Karyotype = 5 AA m + 2AA sm + 1 AA t + X m + y.

Table 9 Morphometric data for the spermatogonial chromosomes of *Pharoscymnus taoi* Sasaji

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.77 ± 0.0048	3.20 ± 0.0067	4.97	0.1313	13.13	55.31	1.80	Sm
2	1.61 ± 0.0073	2.50 ± 0.0038	4.16	0.1099	10.99	38.70	1.55	m
3	1.50 ± 0.0072	2.58 ± 0.0072	4.08	0.1078	10.78	36.76	1.72	sm
4	1.39 ± 0.0124	2.51 ± 0.0066	3.90	0.1031	10.31	35.64	1.80	sm
5	1.70 ± 0.0065	2.00 ± 0.0065	3.70	0.0978	09.78	45.95	1.18	m
6	1.22 ± 0.0075	1.79 ± 0.0081	3.01	0.0795	07.95	40.53	1.46	m
7	1.15 ± 0.0077	1.80 ±0.0058	2.95	0.0780	07.80	38.95	1.56	m
8	0.98 ± 0.0152	1.50 ± 0.0067	2.48	0.0655	06.55	39.52	1.53	m
9	1.30 ± 0.0066	1.88 ± 0.0073	2.38	0.0629	06.29	54.64	1.44	m
X	2.21 ±0.0067	3.00 ± 0.0075	5.21	0.1380	13.80	42.42	1.36	m
y	1.00 ±0.0048	------------	1.00	0.0264	02.64	-------	----	---

Total length of the genome = 37.84 µm: **2n** = 20; Karyotype = 3 AA sm + 6 AA m + X m + y.

Illeis indica Timberlake (Table 10, plate 7)

The normal chromosomal number was confirmed the typical 2n = 20. Karyotype analysis had the following break up: 5 AA submetacentric (sm), 4 AA telocentric (t) and only the longer X chromosome appeared to be the metacentric. As usually y was dot shaped and measured 0.98 µm in length. The mean total length of the individual chromosome varied from 4.43 µm. (largest) to 2.5 µm in autosomes and 4.46 µm in X chromosome. The relative length ranged from 0.12 to 0.07. Arm ratio and the centromeric indices varied in between 2.14 to 1.37 and 43.72 to 31.18 respectively (Table 10. and plate 7).

Psyllobora bisoctonotata (Mulsant) (Table 11, plate 8)

Instead of the typical formate, here 2n= 18, of which 5 pairs of AA were sm whose arm ratios ranged from 1.81 to 1.7; 3 pairs of AA were metacentric type arm ratios of which ranged from 1.63 to 1.5 and the X chromosome was also metacentric with 1.25 arm ratio. Typically the y was too small, measured only 0.95µm in length with no centromeric indication. Also X was the largest with 5.4 µm in length and the relative length and the centromeric index were 0.16 and 44.44 respectively. Of the autosomes the ratios of relative length and the centromeric indices ranged from 0.12 to 0.07 and 39.75 to 35.32 respectively (Table 11 and plate 8).

Cheilomenes sexmaculata (Fabricius) (Table 12, plate 9)

It exhibited a consistent diploid number of also 20.The karyotypic analysis revealed that all the autosomes and the X chromosome were metacentric (m) while the y chromosome was dot shaped and measured only 0.96 µm. Like all the other species , X measured longest in their length which was 4.71 µm. Mean total length of the autosome varied from 3.85 µm to 2.09 µm (Table 12 and plate 9).

PLATE -7

Illeis indica Timberlake

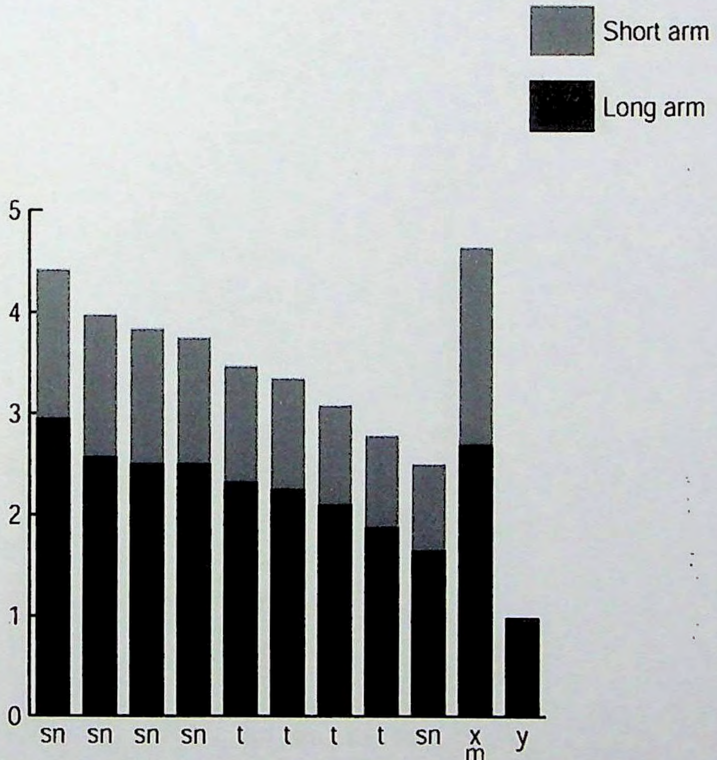
FIG- A

Metaphase chromosomes of *Illeis indica* Timberlake

FIG- B

Karyotype of the metaphase chromosomes of *Illeis indica* Timberlake

FIG- C



The Idiogram in spermatogonial cell of *Illeis indica* Timberlake

FIG- D

PLATE -8

Psyllobora bisoctonotata (Mulsant)

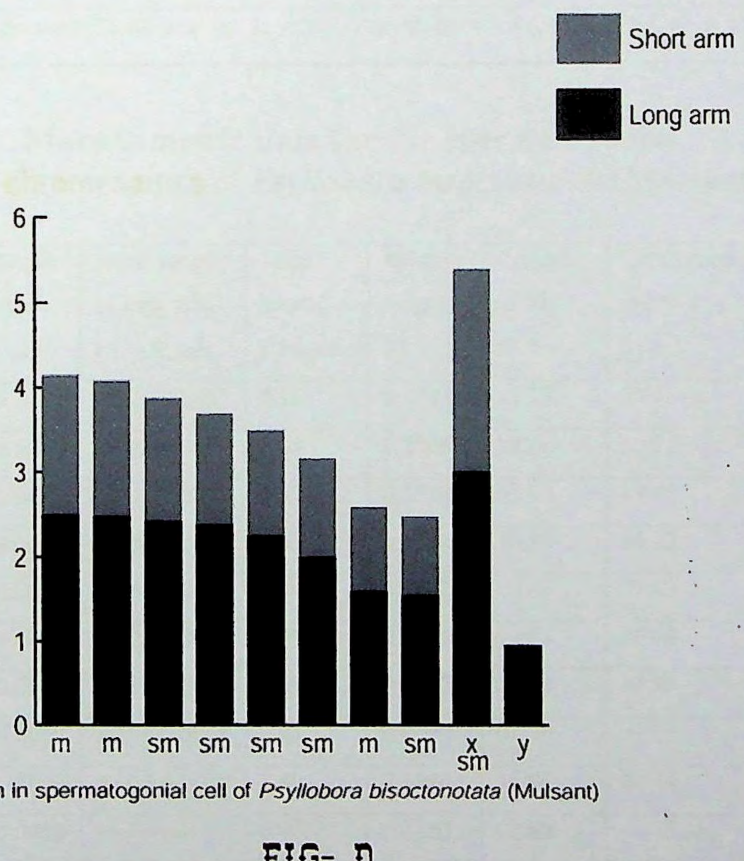
FIG- A

Metaphase chromosomes of *Psyllobora bisoctonotata* (Mulsant)

FIG- B

Karyotype of the metaphase chromosomes of *Psyllobora bisoctonotata* (Mulsant)

FIG- C



The Idiogram in spermatogonial cell of *Psyllobora bisoctonotata* (Mulsant)

FIG- D

Table 10 Morphometric data for the spermatogonial chromosomes of *Illeis indica* Timberlake

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.48 ± 0.0063	2.95 ± 0.0075	4.43	0.1210	12.10	33.41	1.99	sm
2	1.41 ± 0.0143	2.57 ±0.0017	3.98	0.1087	10.87	35.43	1.82	sm
3	1.34 ± 0.0032	2.50 ± 0.0094	3.84	0.1049	10.49	34.81	1.86	sm
4	1.25 ± 0.0053	2.50 ± 0.0061	3.75	0.1024	10.24	33.33	2.00	sm
5	1.15 ± 0.0082	2.32 ± 0.0143	3.47	0.0948	09.48	33.14	2.02	t
6	1.10 ± 0.0075	2.25 ± 0.0078	3.35	0.0915	09.15	32.84	2.04	t
7	0.98 ± 0.0084	2.10 ± 0.0082	3.08	0.0841	08.41	31.18	2.14	t
8	0.90 ± 0.0152	1.88 ± 0.0054	2.78	0.0759	07.59	32.37	2.08	t
9	0.85 ± 0.0161	1.65 ± 0.0081	2.5	0.0683	06.83	34.00	1.94	sm
X	1.95 ± 0.0054	2.69 ±0.0075	4.46	0.1218	12.18	43.72	1.37	m
Y	0.98 ± 0.0062	------------	0.98	0.0268	02.68	------	-----	---

Total length of the genome = 36.62 µm. : 2n = 20. ; Karyotype = 5 AA sm + 4 AA t + X m + y.

Table 11 Morphometric data for the spermatogonial chromosomes of *Psyllobora bisoctonotata*(Mulsant)

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.65 ± 0.0042	2.50± 0.0055	4.15	0.1227	12.27	39.75	1.50	m
2	1.60 ± 0.0051	2.48± 0.0071	4.08	0.1205	12.06	39.21	1.55	m
3	1.45 ± 0.0052	2.42± 0.0056	3.87	0.1144	11.44	37.46	1.70	sm
4	1.30 ± 0.0066	2.38± 0.0058	3.68	0.1088	10.88	35.32	1.83	sm
5	1.24 ± 0.0058	2.25± 0.0066	3.49	0.1032	10.32	35.53	1.81	sm
6	1.15 ± 0.0055	2.00± 0.0073	3.15	0.0931	09.31	36.50	1.73	sm
7	0.98 ± 0.0073	1.60± 0.0056	2.58	0.0762	07.62	37.98	1.63	m
8	0,92 ± 0.0060	1.55± 0.0068	2.47	0.0730	07.30	37.25	1.70	sm
X	2.40 ±0.0057	3.00± 0.0072	5.40	0.1596	15.96	44.44	1.25	m
y	0.95 ± 0.0043	------------	0.95	0.0280	02.80	---------	------	

Total length of the genome = 33. 82 µm : 2n = 18 ; Karyotype = 3 AA m + 5 AA Sm + X m + y.

Coccinella septempunctata Linnaeus (Table 13, plate 10)

The analysis of the chromosomes of this species of coccinellid revealed a diploid number of 20 like the previous member of the genus *Coccinella* . The karyotypic analysis exhibited the following break up: 15 sub-metacentric (sm) including X chromosome, 2 pairs metacentric (m) and 1 pair telocentric (t) with arm ratio 2.95. X was largest chromosome of the complement measured 5.99 μ m in length. Like the others y was smallest chromosome measured only 1.17 μ m (Table 13 and plate 10). The largest length of the autosomes was 5.1μm and relative length value ranged from highest 0.14. to the lowest 0.06. Mean length of the total genome was 40.83 μm. Of the AA No. 5 and No. 8 chromosomes showed 'V' and 'J' shaped respectively while the X was submetaecntric as well as acrocentric type.

Coccinella transversalis Fabricius (Table 14, plate 11)

In this species the diploid set showed 2n= 20. Normal karyotypes included all the 9 pairs metacentric autosomes and a sub-metacentric X chromosome with a dumble shaped y chromosome. The quantitative characters showed a structural range of the mean total length of the arms of autosomes between 3.92 μm to 2.09 μm. while that of the X chromosome was 5.8 μ m and y showed only 1.6 μ m. The relative length of the autosomes ranged between 0.11 to 0.06 as well as the arm ratio and the centromeric indices of those vary from 1.32 to 1.05 and 48.69 to 43.67 respectively The relative length of the X chromosome was 0.16. as well as the arm ratio and the centromeric index were 1.66 and 37.59 respectively. The whole complements were metacentric type and the X chromosome was rod shaped.

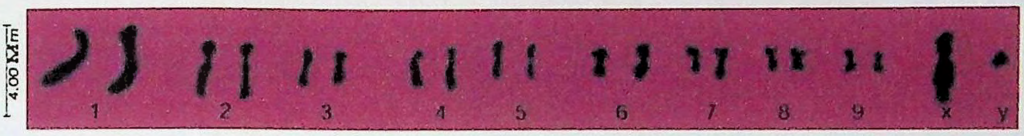
PLATE -9

Cheilomenes sexmaculata (Fabricius)

FIG- A

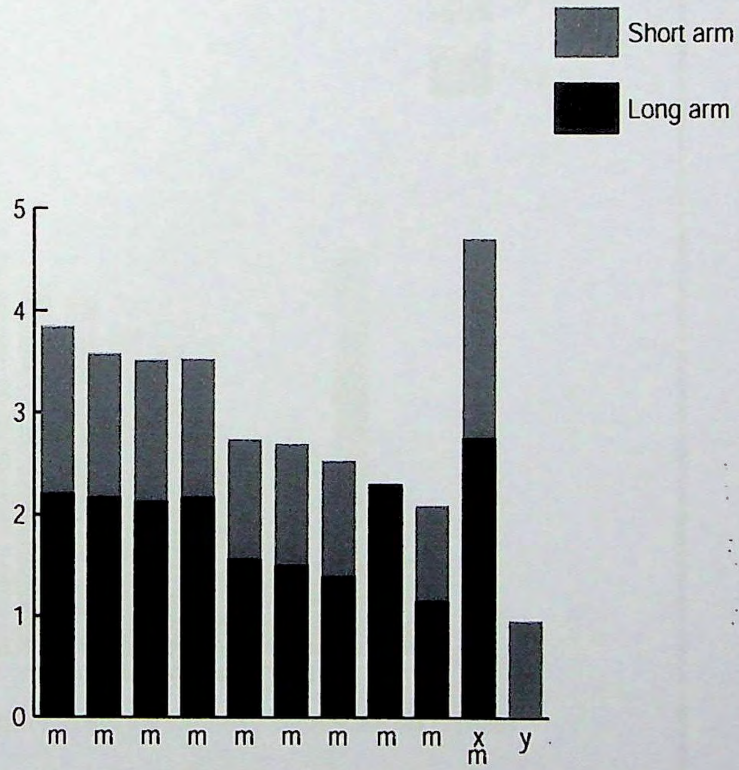
Metaphase chromosomes of
Cheilomenes sexmaculata (Fabricius)

FIG- B



Karyotype of the metaphase chromosomes of *Cheilomenes sexmaculata* (Fabricius)

FIG- C



The Idiogram in spermatogonial cell of *Cheilomenes sexmaculata* (Fabricius)

FIG- D

PLATE -10

Coccinella septempunctata Linnaeus

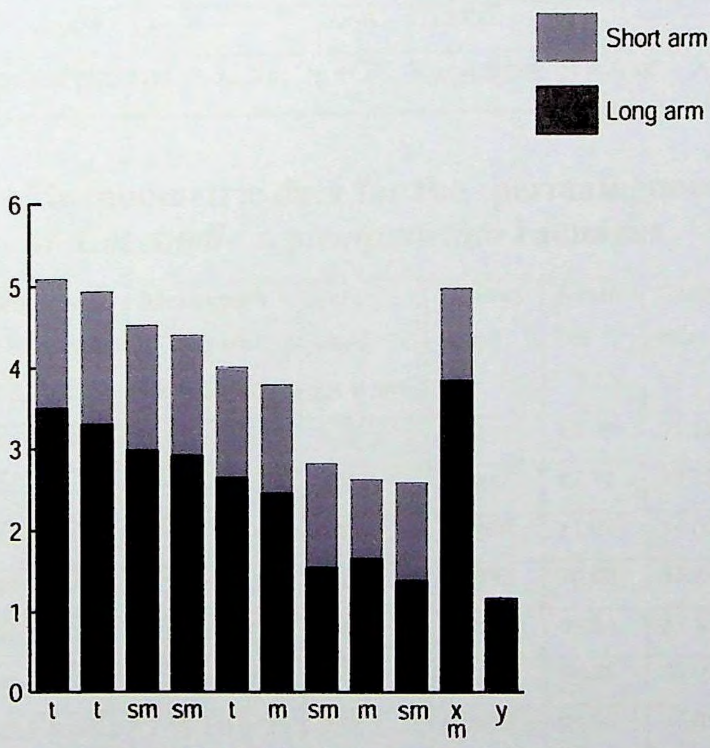
FIG- A

Metaphase chromosomes of *Coccinella septempunctata* Linnaeus

FIG- B

Karyotype of the metaphase chromosomes of *Coccinella septempunctata* Linnaeus

FIG- C



The Idiogram in spermatogonial cell of *Coccinella septempunctata* Linnaeus

FIG- D

Table 12 Morphometric data for the spermatogonial chromosome of *Cheilomenes sexmaculata* (Fabricius)

Chrom Pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Centromeric type
1	1.64 ± 0.0054	2.21 ± 0.0053	3.85	0.1205	12.05	42.6	1.35	m
2	1.41 ± 0.0056	2.17 ± 0.0043	3.58	0.1120	11.20	39.39	1.54	m
3	1.39 ± 0.0056	2.13 ± 0.0175	3.52	0.1102	11.02	39.49	1.53	m
4	1.36 ± 0..0064	2.17 ± 0.0146	3.53	0.1130	11.30	38.53	1.59	m
5	1.17 ± 0.0061	1.57 ± 0.0059	2.72	0.0851	08.51	43.01	1.34	m
6	1.18 ± 0.0119	1.51 ± 0.0132	2.69	0.0842	08.42	43.87	1.28	m
7	1.13 ± 0.0119	1.40 ± 0.0065	2.53	0.0792	07.92	44.66	1.24	m
8	1.03 ± 0.0133	1.27 ± 0.0163	2.30	0.0724	07.24	44.78	1.23	m
9	0.93 ± 0.0141	1.16 ± 0.0138	2.09	0.0654	06.54	44.50	1.25	m
X	1.96 ± 0.0065	2.75 ± 0.0243	4.71	0.1475	14.75	41.61	1.40	m
y	0.96 ± 0.0074	-------------	0.96	0.0300	03.00	-------	-----	

Total length of the genome = 32.48: 2n = 20: Karyotype: 9 AA m + X m + y:

Table 13 Morphometric data for the spermatogonial chromosomes of *Coccinella septempunctata* Linnaeus

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	percent of RL RL%	Centromeric index CI	Arm ratio AR	Centrmeric type
1	1.59 ± 0.0071	3.51 ± 0.0102	5.10	0.1249	12.49	31.18	2.21	t
2	1.64 ± 0.0057	3.31 ± 0.0058	4.95	0.1212	12.12	33.13	2.02	t
3	1.54 ± 0.0127	2.99 ± 0.0109	4.53	0.1109	11.09	34.00	1.94	sm
4	1.49 ± 0.0086	2.92 ± 0.0373	4.41	0.1080	10.80	33.79	1.96	sm
5	1.36 ± 0.0065	2.65 ± 0.0071	4.02	0.0984	09.84	33.83	2.95	t
6	1.33 ± 0.0135	2.46 ± 0.0062	3.79	0.0928	09.28	35.09	1.85	sm
7	1.27 ± 0.0072	1.55 ± 0.0224	2.82	0.0890	08.90	45.03	1.22	m
8	0.97 ± 0.0088	1.66 ± 0.0111	2.63	0.0644	05.44	36.88	1.71	sm
9	1.20 ± 0.0073	1.39 ± 0.0097	2.59	0.0634	06.34	46.33	1.16	m
X	2.14 ± 0.0638	3.85 ± 0.0065	5.99	0.1467	14.67	35.37	1.80	sm
y	1.17 ± 0.0075	------	1.17	0.0278	02.78	-------	-----	---

Total length of the genome = 42.00 µm; 2n = 20; Karyotyope = 3 AA tt + 4 AA Sm + 2 AA m + X Sm + y.

Harmonia octomaculata (Fabricius) (Table 15, plate 12)

The analysis of the chromosome of this species revealed a diploid number of 16 unlike the typical number of the family Coccinellidae. The karyotypic analysis showed that all the autosomes were metacentric and their arm ratios varied from 1.29 to 1.6. The mean total length of the AA measured 4.45 μm in highest and 2.99 μm in lowest The percentage of relative length ranged from 13.5 to 9.1 as well as their centromeric indices from 41.57 to 38.46. Like the other X chromosome also measured highest in length which was 5.29 μ m and y also too small to calculate the arm ratio.It was only 1.4 μm in length. The X exhibited sub-metacentric (sm.) type as its value of arm ratio was1.89 (Table 15.and plate 12).

Micraspis discolor Fabricius(Table 16, plate 13)

It exhibited a consistent diploid number of 20. The karyotypic analysis revealed that the autosomes were 6 pairs sub-metacentric and rest 3 pairs are metacentric while of the sex chromosomes X was sub-metacentric and conspiciously large. The y chromosome was, however, smallest of the complement and without distinct centromere, measured only 0.9 μm and X measured 5.95 μm . The mean total length of the autosomes varied from 5.29 μm to 1.96 μm (Table 16 and plate 13). The relative length showed a range between 0.16 to 0.05. Arm ratio of the metacentric chromosome were between 1.44 to 1.69 and that of the sub- metacentric were between 1.74 to 2.28. The centromeric indices ranged within 41.12 to 31.76.

Micraspis yasumatsui Sasaji (Table 17, plate 14)

In this common lady bird beetle, the diploid set showed the typical chromosome number 2n =20. Normal karyotypes included 2 pairs of Autosomes (AA) sub-metacentric (sm) arm ratios of which are 1.93 & 1.87 and the rest 7 pairs of AA metacentric type, and their arm ratios varied between 1.2 to 1.49. Of the sex chromosome X is sub-metacentric and y is as usual minuten centromere of

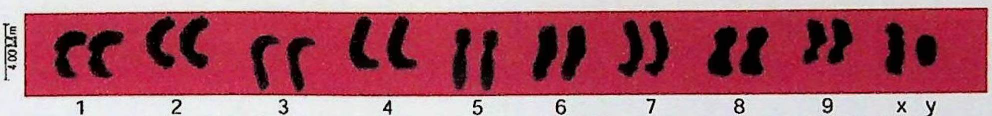
PLATE -11

Coccinella transversalis (Fabricius)

FIG- A

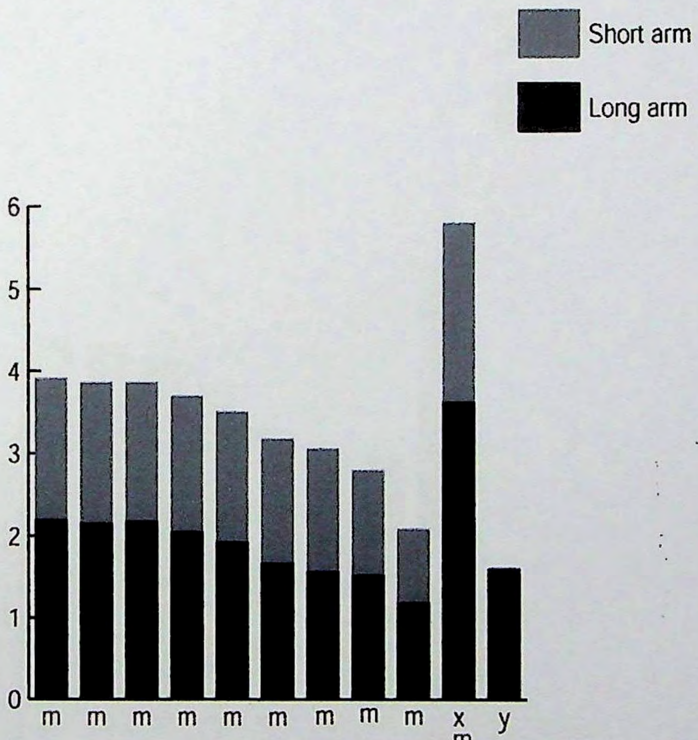
Metaphase chromosomes of
Coccinella transversalis (Fabricius)

FIG- B



Karyotype of the metaphase chromosomes of *Coccinella transversalis* (Fabricius)

FIG- C



The Idiogram in spermatogonial cell of *Coccinella transversalis* (Fabricius).

FIG- D

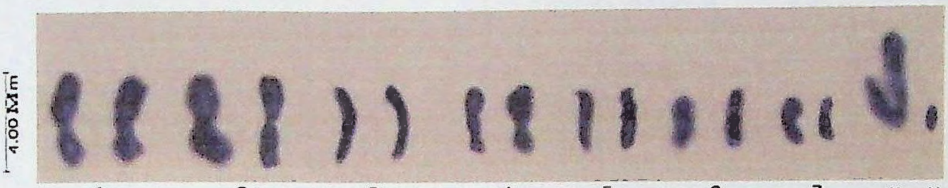
PLATE -12

Harmonia octomaculata (Fabricius)

FIG- A

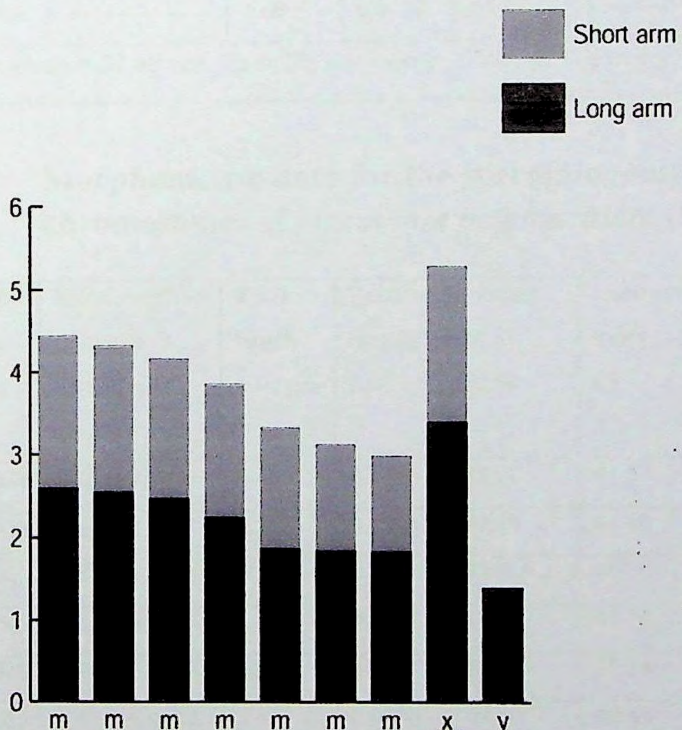
Metaphase chromosomes of *Harmonia octomaculata* (Fabricius)

FIG- B



Karyotype of the metaphase chromosomes of *Harmonia octomaculata* (Fabricius)

FIG- C



The Idiogram in spermatogonial cell of *Harmonia octomaculata* (Fabricius)

FIG- D

Table 14 Mphometric data for the spermatogonial chromosomes of *Coccinella transversalis* Fabricius

Chrom pair	Mean length of short arm s ± S.E.µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent, of RL RL%	Centromeric index CI	Arm ratio AR	Centromeric type
1	1.72 ± 0.0092	2.20 ± 0.0253	3.92	0.1095	10.95	43.88	1.27	m
2	1.71 ± 0.0082	2.16 ± 0.0231	3.87	0.1081	10.81	44.19	1.26	m
3	1.69 ± 0.0024	2.18 ± 0.0167	3.87	0.1081	10.81	43.67	1.28	m
4	1.65 ± 0.0076	2.05 ± 0.0230	3.70	0.1033	10.33	44.59	1.24	m
5	158 ± 0.0071	1.93 ± 0.0079	3.51	0.0980	09.80	45.01	1.22	m
6	1.51 ± 0.0066	1.67 ± 0.0143	3.18	0.0888	08.88	47.48	1.10	m
7	1.49 ± 0.0067	1.57 ± 0.0122	3.06	0.0855	08.55	48.69	1.05	m
8	1.27 ± 0 0174	1.53 ± 0.0153	2.80	0.0782	07.82	45.36	1.20	m
9	0.90 ± 0.0158	1.19 ± 0.0093	2.09	0.0584	05.84	44.33	1.32	m
X	2.18 ± 0.0095	3.62 ± 0.0157	5.80	0.1620	16.20	37.59	1.66	m
y	1.6 ± 0.0058	--------------	1.60	0.0427	04.27	-------	-----	---

Total length of the genome = 37.40 µm; 2n = 20; Karyotype: 9 AA m + X m + y.

-

Table 15 Morphometric data for the spermatogonial chromosomes of *Harmonia octomaculata* (Fabricus)

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.85 ± 0.0074	2.60 ± 0.0076	4.45	0.1350	13.50	41.57	1.40	m
2	1.78 ± 0.0062	2.55 ± 0.0057	4.33	0.1314	13.14	41.10	1.43	m
3	1.70 ± 0.006	2.47 ± 0.0048	4.17	0.1266	12.66	40.76	1.45	m
4	1.61 ± 00054	2.25 ± 0.0045	3.86	0.1162	11.62	41.70	1.39	m
5	1.45 ± 0.0048	1.88 ± 0.0056	3.33	0.1011	10.11	43.54	1.29	m
6	1.28 ± 0.0055	1.85 ± 0.0082	3.13	0.0950	09.50	40.89	1.45	m
7	1.15 ± 0.0065	1.84 ± 0.0053	2.99	0.0910	09.10	38.46	1.60	m
X	1.89 ± 0.0056	3.40 ± 0.0064	5.29	0.1605	16.05	35.72	1.89	sm
y	1.40 ± 0.0047	--------------	1.4	0.0425	04.25	-----	------	----

Total length of the genome = 32.95 µm . : 2n = 16 (7 AA + X y) Karyotype = 7 AA m + X sm +y.

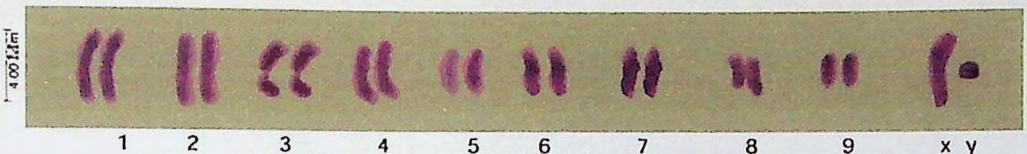
PLATE -13

Micraspis discolor Fabricius

FIG- A

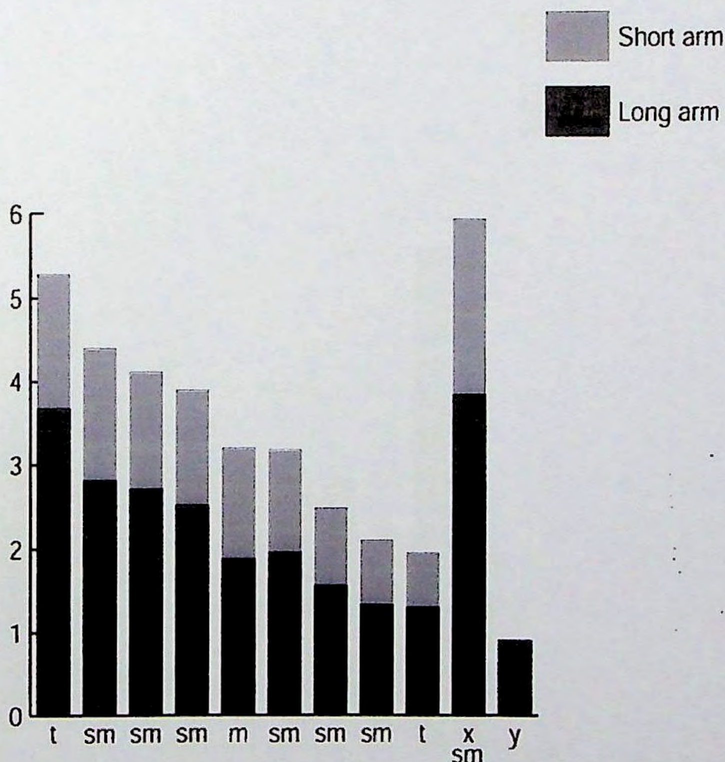
Metaphase chromosomes of *Micraspis discolor* Fabricius

FIG- B



Karyotype of the metaphase chromosomes of *Micraspis discolor* Fabricius

FIG- C



The Idiogram in spermatogonial cell of *Micraspis discolor* Fabricius

FIG- D

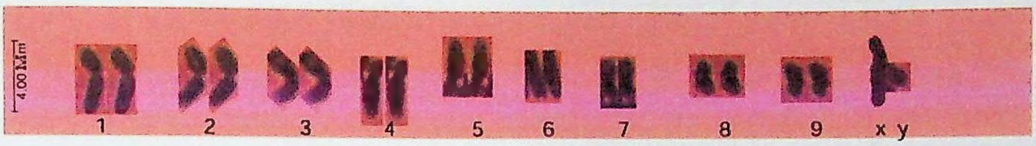
PLATE -14

Micraspis yasumatsui Sasaji

FIG- A

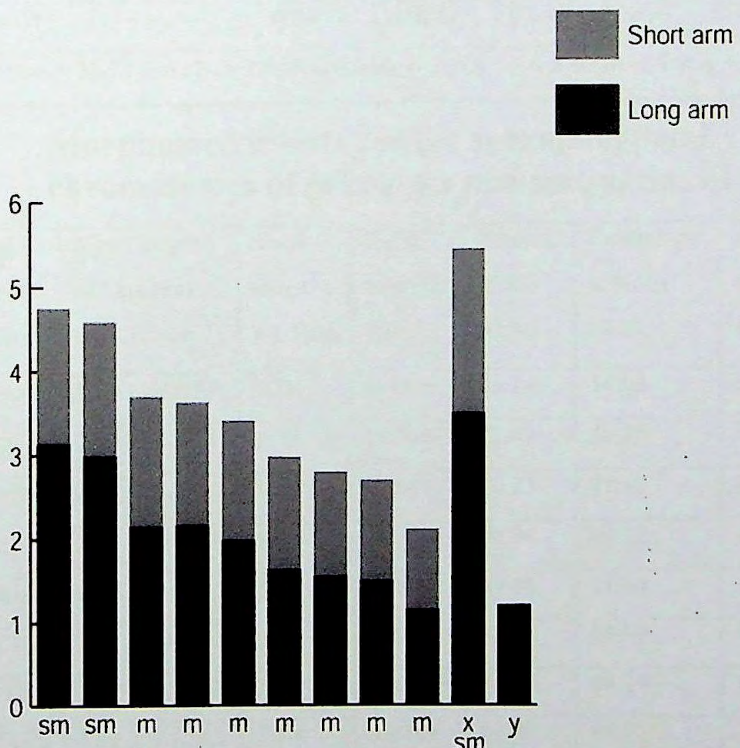
Metaphase chromosomes of *Micraspis yasumatsui* Sasaji

FIG- B



Karyotype of the metaphase chromosomes of *Micraspis yasumatsui* Sasaji

FIG- C



The Idiogram in spermatogonial cell of *Micraspis yasumatsui* Sasaji

FIG- D

Table 16 Morphometric data for the spermatogonial chromosomes of *Micraspis discolor* Fabricius

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centro meric index CI	Arm ratio AR	Cenro meric type
1	1.61 ± 0.0057	3.68± 0.0102	5.29	0.1441	14.41	31.76	2.28	t
2	1.58 ± 0.0076	2.82± 0.0182	4.39	0.1195	11.95	35.99	1.78	sm
3	1.40 ± 0.0099	2.72± 0.0137	4.21	0.1147	11.47	35.39	1.82	sm
4	1.37 ± 0.0105	2.53± 0.0045	3.90	0.1062	10.62	35.13	1.85	sm
5	1.32 ± 0.0070	1.89± 0.0053	3.21	0.0874	08.74	41.12	1.44	m
6	1.22 ± 0.0107	1.97± 0.0094	3.19	0.0869	08.69	38.24	1.71	sm
7	0.93 ± 0.0118	1.57± 0.0070	2.50	0.0681	06.81	37.20	1.69	sm
8	0.77 ± 0.0143	1.34± 0.0152	2.11	0.0575	05.75	36.49	1.74	sm
9	0.65 ± 0.0086	1.31± 0.0146	1.96	0.0539	05.39	33.16	2.10	t
X	2.10 ± 0.0187	3.85± 0.0086	5.95	0.1621	16.21	35.29	1.83	sm
y	0.91 ±0.0084	-----------	0.91	0.0247	02.47	------	------	

total loength of the genome = 36.71 µm.:2n = 20.; Karyotype = 2 AA t + 6 AA sm + 1 AA + X sm + y.

Table 17 Morphometric data for the spermatogonial chromosomes of *Micraspis yasumatsui* Sasaji

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenro meric type
1	1.62 ± 0.0042	3.14 ± 0.0187	4.76	0.1316	13.16	34.03	1.93	sm
2	1.60 ± 0.0077	2.99 ± 0.0371	4.59	0.1369	12.69	34.46	1.87	sm
3	1.55 ± 0.0085	2.15 ± 0.0267	3.70	0. 1023	10.23	41.49	1.47	m
4	1.46 ± 0.0172	2.17 ± 0.0145	3.63	0.1004	10.04	40.22	1.49	m
5	1.42 ± 0.0164	1.99 ± 0.0261	3.41	0.0943	09.43	41.64	1.40	m
6	1.34 ± 0.0100	1.64 ± 0.0098	2.98	0.0824	08.24	44.97	1.22	m
7	1.24 ± 0.0076	1.56 ± 0.0134	2.80	0.0774	07.74	44.29	1.25	m
8	1.19 ± 0.0079	1.51 ± 0.0272	2.70	0.0747	07.47	44.07	1.26	m
9	0.96 ± 0.0058	1.16 ± 0.0164	2.12	0.0671	06.71	45.28	1.20	m
X	1.96 ± 0.0074	3.51 ± 0.0226	5.47	0.1513	15.13	35.83	1.79	sm
y	1.21 ± 0.0065	-------------	1.21	0.0335	03.35	---------	-----	----

Total loength of the genome = 36.16 µm : 2n = 20 ; Karyotype = 2 AA sm + 7 AA m + X sm + y.

which was indistinct as well as the total length of it is only 1.2 µm. The value of arm ratio of X chromosome was 1.79. The largest X measured 5.47 µm in length. The quantitative characters of the AA showed a structural range of the of the mean total length of the arms between 4.76 µm to 2.12 µm (Table 17 & plate 14).The relative length of the chromosomes had a range of 0.15 to 0.076 and the centromeric indices ranged from 44.97 to 34.03.

Propylea quatuordecimpunctata Linneaus(Table 18, plate 15)

The diploid chromosome number assigned to this smaller species is 20 as revealed by the majority of the chromosome plates. The karyotype was made by arranging the chromosome in gradual series of length rather arbitrarily. The chromosome had the following break up : of the autosomes (AA) 4 pairs sm, 3 pairs m and the 2 pairs t while of the sex chromosome X was also m and y was without distinct centromere. The morphometric data are appended in table 18 and karyotype in Plate 15. The table showed the mean total length of the autosomes varied between 4.07 µm to 2.42 µm while the sex chromosomes measured to be 5.1µm, for X and 1.00 µm for y chromosome. The relative length ranged from 0.14. to 0.07 in the whole complement. The arm ratio and the centromeric indices of the submetacentric chromosome were between 1.98 to 1.78 and 35.93 to 34.89 respectively. The metacentric pairs amongst the autosomes had the centromeric indices 38.66 to 37.27 whose arm ratios were 1.68, 1.63 and 1.59 respectively while those of the other two telocentric pairs were centromeric indices 35.67 and 33.0 as well as the arm ratio 2.1 in both. X chromosome with the value of 1.55 for arm ratio 39.21 for centromeric index was the largest of the whole complement and y like the other was the smallest.

* *Apomycraspis quayumi* Ali & Rahaman(Table 19, plate 16)

Analysis of the chromosome preparation revealed a consistent diploid number of 20 chromosomes having no deviation from the 2n value. The karyotype analysis confirmed that the steadily decreasing autosomes were 2 pairs metacentric, 4 pairs were sub-metacentric and 3 pairs were telocentric while of the sex chromosomes, X was metacentric and y was so small that the presence of centromere was indistinct. The mean length of each chromosome pair alongwith other morphometric data viz relative length, arm ratio and centromeric index are given in table 19. and its karyotypes are in the plate 16.

The autosome ranged in length from 4.75 µm to 2.45 µm As about the sex chromosome the hemizygous condition was almost universal. The mean length of the X chromosome was 5.35 µm and that of the y chromosome was 1.15 µm The relative length ranged from 0.11to 0.06 in case of autosomes and it was 0.13 in X chromosome while 0.032 in y chromosome. The centromeric indices had a series between 42.05 to 31.11. The arm ratio of the chromosome pairs 1-4 were 1.89 to 1.92 which were truly sub-metacentric, Nos. 5-7 showed the AR value were 2.21 to 2.01 which indicated telocentric type and the rest were metacentric.

Afidenta misera Mulsant (Table 20, plate 17)

The analysis of the chromosome of this species revealed a diploid number of 14 unlike any of other members of this family. The karyotype analysis exhibited the folllowing break up: 3 pairs of AA metacentric with the arm ratio 1.64 to 1.51 and the value of centromeric indices were 39.73 to 37.11. The rest 3 pairs of AA were sm with the arm ratio 2.02 to 1.78 and the value of centromeric indices were 35.91 to 32.43. X was also sm. with the AR 1.76 and the value of CI 36.23. Mean total of the genome was 30.31µm of which the mean total length of the individual chromosome varied from 5.41µm to 2.97 µm excluding y chromosome which was smallest with only 0.98 µm without distinct chromosome. RL value ranged from 0.18 to 0.10
(Table 20 and plate 17).

Epilachna septima Mulsant (Table 21, plate 18)

The normal diploid number was 20. The karyotype analysis had the following break up: 4 pairs of AA sm , 1 pair t and 4 pairs m on the other hand of the sex chromosome

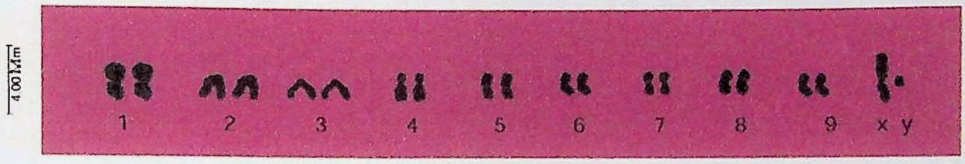
PLATE -15

Propylea quatuordecimpunctata Linnaeus

FIG- A

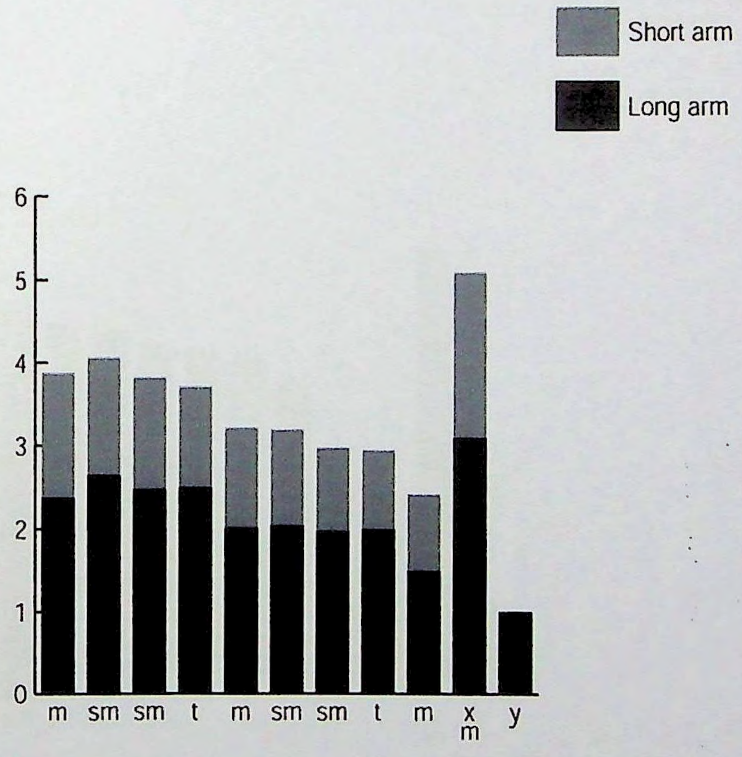
Metaphase chromosomes of *Propylea quatuordecimpunctata* Linnaeus

FIG- B



Karyotype of the metaphase chromosomes of *Propylea quatuordecimpunctata* Linnaeus

FIG- C



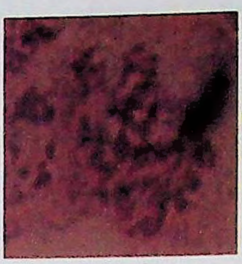
The Idiogram in spermatogonial cell of *Propylea quatuordecimpunctata* Linnaeus

FIG- D

PLATE -16

Apomicraspis quayumi Ali & Rahman

FIG- A

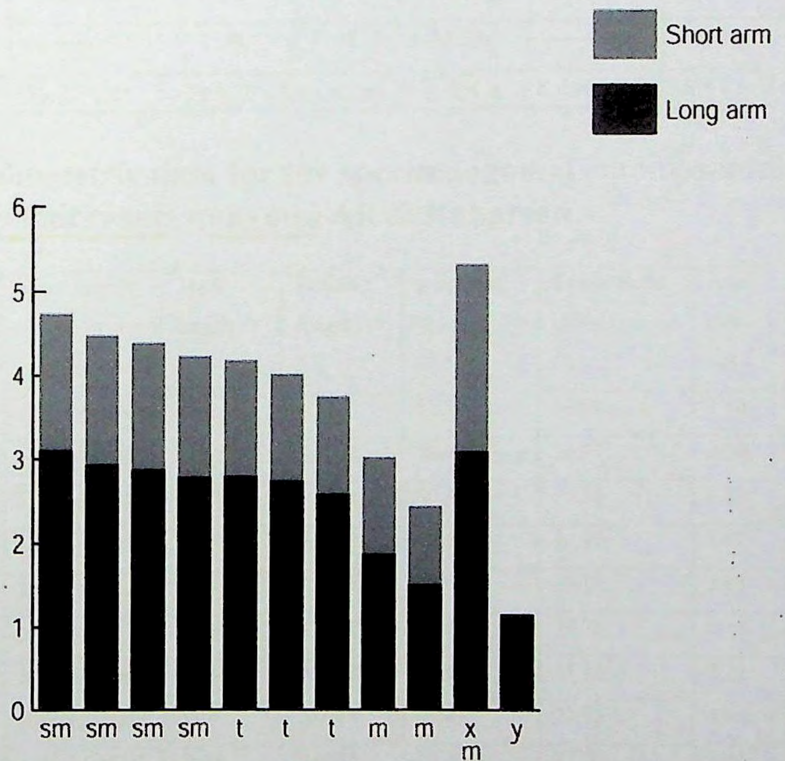


Metaphase chromosomes of *Apomicraspis quayumi* Ali & Rahman

FIG- B

Karyotype of the metaphase chromosomes of *Apomicraspis quayumi* Ali & Rahman

FIG- C



The Idiogram in spermatogonial cell of *Apomicraspis quayumi* Ali & Rahman

FIG- D

Table 18 Morphometric data for the spermatogonial chromosomes of *Propylea quatuordecimpunctata* Linnaeus

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.50 ± 0.0065	2.38± 0.0162	3.88	0.1067	10.67	38.66	1.59	m
2	1.42 ± 0.0073	2.65± 0.0072	4.07	0.1119	11.19	34.89	1.87	sm
3	1.35 ± 0.0153	2.48± 0.0066	3.83	0.1053	10.53	35.25	1.83	sm
4	1.22 ± 0.0076	2.50± 0.0074	3.72	0.1022	10.22	35.67	2.10	t
5	1.20 ± 0.0075	2.02± 0.0154	3.22	0.0885	08.85	37.27	1.68	m
6	1.15 ± 0.0066	2.05± 0.0077	3.20	0.0880	08.80	35.93	1.78	sm
7	1.00 ± 0.0132	1.98± 0.0068	2.98	0.0819	08.19	33.55	1.98	sm
8	0.95 ± 0.0084	2.00± 0.0075	2.95	0.0811	08.11	33.90	2.10	t
9	0.92 ±0.0065	1.50± 0.0073	2.42	0.0665	06.65	38.00	1.63	m
X	2.00 ±0.0163	3.10± 0.0057	5.10	0.1402	14.02	39.21	1.55	m
Y	1.00 ± 0.0074	------------	1.00	0.0275	02.75	------	------	-----

Total length of the genome = 36.37 µm : 2n =20 ; Karyotype = 3 AA m + 4 AA sm + 2 AA t + X m + y.

Table 19 Morphometric data for the spermatogonial chromosomes of Apomicraspis quayumi Ali & Rahaman

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	percent of RL RL%	Centromeric index CI	Arm ratio AR	Centromeric type
1	1.64 ± 0.0067	3.11 ± 0.0065	4.75	0.1135	11.35	33.68	1.90	sm
2	1.55 ± 0.0071	2.94 ± 0.0066	4.49	0.1072	10.72	34.52	1.90	sm
3	1.52 ± 0.0054	2.88 ± 0.0125	4.40	0.1051	10.51	34.54	1.89	sm
4	1.45 ± 0.0073	2.79 ± 0.0054	4.24	0.1013	10.13	34.19	1.92	sm
5	1.39 ± 0.0082	2.80 ± 0.0072	4.19	0.1001	10.01	33.17	2.01	t
6	1.28 ± 0.0051	2.75 ± 0.0055	4.03	0.0963	09.63	31.76	2.14	t
7	1.17 ± 0.0066	2.59 ± 0.0064	3.76	0.0898	08.98	31.11	2.21	t
8	1.15 ± 0.0154	1.88 ± 0.0067	3.03	0.0724	07.24	37.95	1.63	m
9	0.93 ± 0.0132	1.52 ± 0.0073	2.45	0.0585	05.85	37.96	1.63	m
X	2.25 ±0.0095	3.10 ± 0.0125	5.35	0.1278	12.78	42.05	1.38	m
y	1.15 ±0.0073	-----	1.15	0.0274	02.74	-----	----	

Total length of the genome = 41.85 µm : 2n = 20; Karyotype = 4 AA sm + 3 AA t + 2 AA m + X m + y.

X was also metacentric and y was the smallest without distinct centromere. The mean total length of the individual chromosome varied from 5.1 μm to 0.95 μm of which highest was for the X and lowest for the y. Autosome were in between 4.5 μ m to 2.01 μm. The relative length ranged from 0.13. to 0.05. The arm ratio of the metacentric chromosomes had a drift between 1.65 to 1.28 whose centromeric indices ranged between 43.78 to 37.7. These measurements in case of submetacentric chromosomes were 1.82 to 1.77 and 36.56 to 35.35 respectively and in telocentric one it was 2.00 and 33.33 respectively. The total length of the genome was 38.37 μm (Table 21). Plate 19 showed the karyotype.

Epilachna pusillanima (Mulsant) (Table 22, plate 19)

In this species 2n =16. The karyotype analysis exhibited 5 pairs of AA sm arm ratio of which were in between 1.81 to 1.72 and the centromeric indices were ranged from 36.62 to 35.51. Rest of the AA as well as the X chromosome were metacentric type and the arm ratio of those were in between 1.7 to 1.4 The CI of the metacentric chromosomes ranged from 41.54 to 36.11. The relative length varied from 0.18 to 0.09 . The total length of the genome was 33.59μm of which AA measured 4.59 μm to 3.24 μm and the X was 5.97 μm while The y was too small with only 0.98μm in length. In the y centromere was indistinct. All the records have showed in the Table 22 and plate 19 .

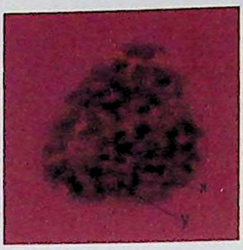
Epilachna vigintioctopunctata (Fabricius) (Table 23, plate 20)

In this species of the lady beetles under the sub family Epilachninae, the diploid set showed very little deviation from the typical 2n= 20 Normal karyotypes included 6 pairs of AA m 2 paires Sm and of the sex chromosome X was also m while y was too small with indistinct centromere. The quantitative characters showed a structural

PLATE -17

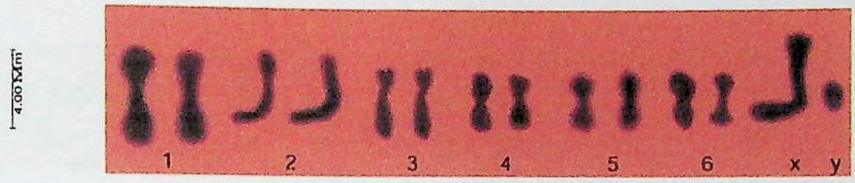
Afidenta misera Mulsant

FIG- A



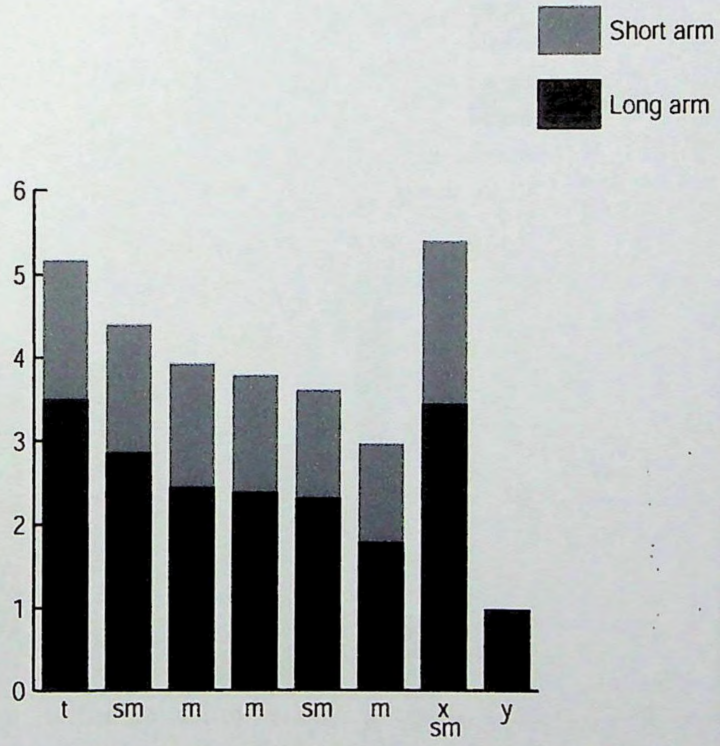
Metaphase chromosomes of *Afidenta misera* Mulsant

FIG- B



Karyotype of the metaphase chromosomes of *Afidenta misera* Mulsant

FIG- C



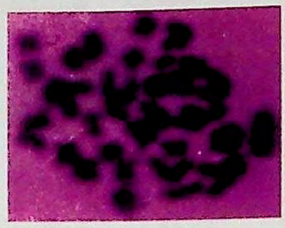
The Idiogram in spermatogonial cell of *Afidenta misera* Mulsant

FIG- D

PLATE -18

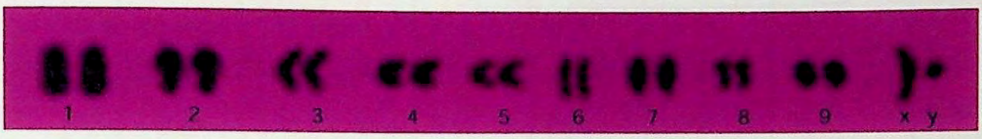
Epilachna septima Mulsant

FIG- A



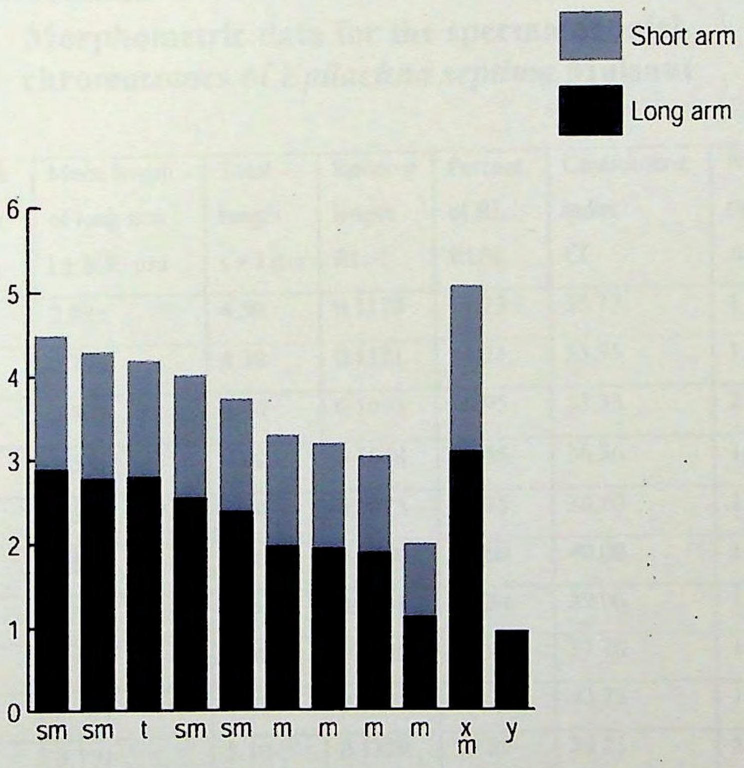
Metaphase chromosomes of *Epilachna septima* Mulsant

FIG- B



Karyotype of the metaphase chromosomes of *Epilachna septima* Mulsant

FIG- C



The Idiogram in spermatogonial cell of *Epilachna septima* Mulsant

FIG- D

Table 20 Morphometric data for the spermatogonial chromosomes of *Afidenta misera* Mulsant

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.68 ± 0.0090	3.50 ± 0.0085	5.13	0.1710	17.10	32.43	2.08	t
2	1.55 ± 0.0063	2.86 ± 0.0052	4.41	0.1455	14.55	35.14	1.84	sm
3	1.49 ± 0.0062	2.45 ± 0.0043	3.94	0.1300	13.00	37.82	1.64	m
4	1.41 ± 0.0045	2.39 ± 0.0051	3.80	0.1254	12.54	37.11	1.69	m
5	1.30 ± 0.0066	2.32 ± 0.0052	3.62	0.1194	11.94	35.91	1.78	sm
6	1.18 ± 0.0050	1.79 ± 0.0058	2.97	0.0980	09.80	39.73	1.51	m
X	1.96 ± 0.0074	3.45 ± 0.0063	5.41	0.1800	18.00	36.23	1.76	sm
y	0.98 ± 0.0080	-------	0.98	0.0323	03.23	--------	----	----

Total length of the genome = 30.31 µm : 2n = 14; Karyotype = 1 AA t + 2 AA m + 2 AA m + X m + y.

Table 21 Morphometric data for the spermatogonial chromosomes of *Epilachna septima* Mulsant

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenromeric type
1	1.61 ±	2.89±	4.50	0.1173	11.73	35.77	1.79	sm
2	1.52±	2.78±	4.30	0.1121	11.21	35.35	1.82	sm
3	1.40±	2.80±	4.20	0.1095	10.95	33.33	2.00	t
4	1.47±	2.55±	4.02	0.1048	10.48	36.56	1.73	sm
5	1.35±	2.39±	3.74	0.0975	09.75	36.10	1.77	sm.
6	1.32±	1.98±	3.30	0.0860	08.60	40.00	1.50	m
7	1.25±	1.95±	3.20	0.0834	08.34	39.06	1.56	m
8	1.15±	1.90±	3.05	0.0795	07.95	37.70	1.65	m
9	0.88±	1.13±	2.01	0.0521	05.21	43.78	1.28	m
X	2.00±	3.10±	5.10	0.1329	13.29	39.21	1.55	m
y	0.95±	------	0.95	0.0247	02.47	--------	---	

Total length of the genome = 38.37 µm: 2n =20; Karyotype = 4 AA sm + 4 AA m + 1 AA t + X m + y.

range of the mean total length of the arms between 6.2µm to 1.95 µm of which X was largest, y was only 1.5 µm in length. (Table 23 and Plate 20). The relative length of the chromosome ranged between 0.12 to 0.04. Arm ratio of the metacentric chromosomes were varied from 1.51 to 1.07 sub-metacentric ones were from 1.96 to 1.79. The centromeric indices ranged from 48.28 to 33.73.

PLATE -19

Epilachna pusillanima (Mulsant)

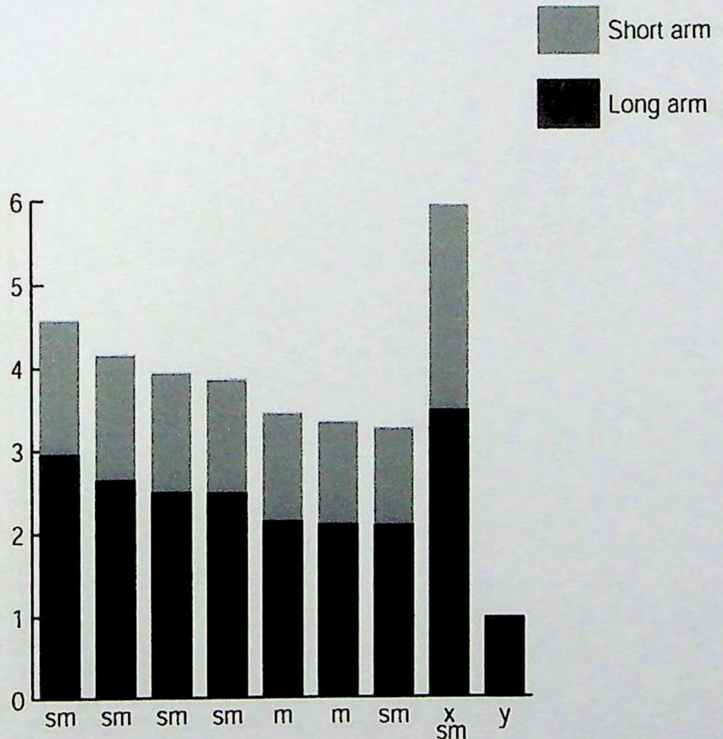
FIG- A

Metaphase chromosomes of *Epilachna pusillanima* (Mulsant)

FIG- B

Karyotype of the metaphase chromosomes of *Epilachna pusillanima* (Mulsant)

FIG- C



The Idiogram in spermatogonial cell of *Epilachna pusillanima* (Mulsant)

FIG- D

PLATE -20

Epilachna vigintioctopunctata (Fabricius)

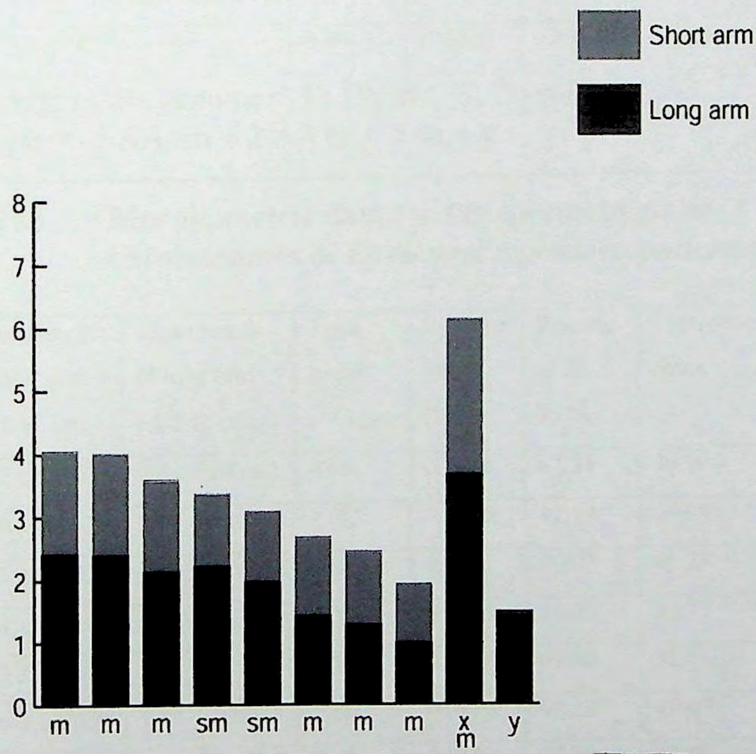
FIG- A

Metaphase chromosome of
Epilachna vigintioctopunctata (Fabricius)

FIG- B

Karyotype of the metaphase chromosome of *Epilachna vigintioctopunctata* (Fabricius)

FIG- C



The Idiogram in spermatogonial cell of *Epilachna vigintioctopunctata* (Fabricius)

FIG- D

Table 22 Morphometric data for the spermatogonial chromosomes of *Epilachna pusillanima* (Mulsant)

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenro meric type
1	1.63 ± 0.0082	2.96 ± 0.0085	4.59	0.1366	13.66	35.51	1.81	sm
2	1.52 ± 0.0143	2.65 ± 0.0073	4.18	0.1244	12.44	36.36	1.74	sm
3	1.45 ± 0.0631	2.50 ± 0.0084	3.96	0.1179	11.79	36.62	1.72	sm
4	1.38 ± 0.0078	2.49 ± 0.0167	3.87	0.1152	11.52	35.65	1.80	sm
5	1.30 ± 0.0092	2.15 ± 0.0145	3.45	0.1027	10.27	37.68	1.65	m
6	1.24 ± 0.0065	2.11 ± 0.0593	3.35	0.0997	09.97	37.02	1.70	m
7	1.17 ± 0.0153	2.10 ± 0.0125	3.24	0.0965	09.65	36.11	1.79	sm
X	2.48 ± 0.0059	3.49 ± 0.0098	5.97	0.1777	17.77	41.54	1.40	m
y	0.98 ± 0.0142	-------	0.98	0.0292	02.92	--------	----	----

Total length of the genome = 33.59µm : 2n = 16
Karyotype = 5 AA sm + 2 AA m + X m + y.

Table 23 Morphometric data for the spermatogonial chromosomes of *Epilachna vigintioctopunctata* (Fabricius)

Chrom pair	Mean length of short arm s ± S.E. µm	Mean length of long arm l ± S.E. µm	Total length s + l µm	Relative length RL	Percent. of RL RL%	Centromeric index CI	Arm ratio AR	Cenro meric type
1	1.62 ± 0.0063	2.44 ± 0.0062	4.06	0.1231	12.31	39.90	1.51	m
2	1.60 ± 0.0076	2.42 ± 0.0097	4.02	0.1219	12.19	39.98	1.51	m
3	1.45 ± 0.0059	2.15 ± 0.0077	3.60	0.1092	10.92	40.28	1.48	m
4	1.14 ± 0.0116	2.24 ± 0.0095	3.38	0.1025	10.25	33.73	1.96	sm
5	1.11 ± 0.0042	1.99 ± 0.0074	3.10	0.0940	09.40	35.81	1.79	sm
6	1.26 ± 0.0050	1.44 ± 0.0065	2.70	0.0819	08.19	46.67	1.14	m
7	1.17 ± 0.0087	1.30 ± 0.0125	2.47	0.0750	07.50	47.37	1.11	m
8	0.94 ± 0.0052	1.01 ± 0.0154	1.95	0.0590	05.90	48.28	1.07	m
X	2.49 ±0.0075	3.70 ± 0.0087	6.20	0.1880	18.80	40.16	1.49	m
y	1.50 ± 0.0082	----	1.50	0.0450	04.50	------	------	----

Total length of the genome =32.98 µm: **2n** = 18; Karyotype = 2 AA sm + 6 AA m + X m + y.

Amino acids in some species of the Coccinellidae

The present investigation also deals with the findings of qualitative analysis of amino acids in favor of cytogetical study of the Coccinellidae. Experimental samples were collected from the body extracts of adult lady beetles at starvation condition.

The samples were hydrolyzed by treating with both acidic and alkaline media. During acid hydrolysis a number of amino acids, especially tryptophan and to a lesser extent serine and threonine are destroyed by prolonged treatment. Alkaline hydrolysis results in the partial or complete destruction of arginine, cystine, serine and threoine; it also causes recemization of remaining amino acid (Clark 1963). For this reasons both acid and alkaline hydrolyses were used in the investigation.

The solvent systems used was n-Butanol : acitic acid : water in the ratio 12 : 3 : 5 . Standard amino acids were run side by side on the TLC plate. The suspected amino acids from the experimental extracts of twelve species of the family Coccinellidae were co- chromatographed with the standard 20 amino acids. The same experiment was done twice. In one, chromatograms were developed in Iodine chamber and next with spraying the ninhydrin solution. Different numbers of spots appeared on the unidimensional plate and the result was calculated to compare the Rf values of unknown samples with the Rf values of standard amino acids. From the total findings mean result of each species are tabulated in the respected tables and photographs are shown in the plates.

Table 24 Rf values of the compounds present *Illeis indica* Timberl with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acids detected
Alanine	0.53	0.55	0.52	++, ++	alanine
Arginine	0.60				
Aspargine	0.40	0.41	0.40	++, +	Aspargine
Aspartic	0.37	0.38		++,	aspartic acid
acid	0.09				
Cystein	0.24		0.23	++	glycine
Glycin	0.49				
Glutamic	0.19				
acid					
Glutamin	0.20				
Histidine	0.59				
Iso-leucine	0.60				
Leucine	0.30				
Lysine	0..63	0.62		++	lysine
Methionine	0.44	0.44	0.29	++	Methionine
Prolin	0.75	0.73	0.74	+	proline
Phenyl-	0.20			++, ++	Phenyl-
alanine					alanine
Serine				+	
Threonine	0.40				
Tryptophan	0.72				
Tyrosine	0.49	0.48		+	tyrosine
Valine	0.65		0.66	+	valine

Amino acids in *Illeis indica* Timberlake (Table 24, plate 21)

There seven spots were appeared on the TLC plate from each of the treatment of ninhydrin as well as iodine reacting compounds in the acid hydrolysate. The Mean Rf values of those 7 spots calculated were 0.55, 0.41 ,0.38, 0.62, 0.44, 0.73, & 0.48 which resembled with the Rf values of the standard amino acids alanine (0.53), aspargine (0.40), aspartic acid (0.37), methionine (0.63), proline (0.44), phenyl-alanine (0.75) and tyrosine (0.49) respectively. On the other hand as the same way, mean Rf values 0.52, 0.40, 0.23, 0.29, 0.74, 0.66 were calculated from the spots appeared in the alkaline hydrolysate correspond with the Rf values of the standard amino acids alanine (0.53), aspargine (0.40), glysine (0.24), lysine (0.30), phenyl-alanine (0.75) and valine (0.66) respectively. The result showed total 13 Rf values from the experimental sample of which 3 were common in both hydrolysate , as for collectively 10 values indicated 10 amino acids alanine, aspargine, aspartic acid, glycine, lysine, methionine, proline, phenyl-alanine, tyrosine and valine present in *Illeis indica* Timberlake. Respective list of the chromatograms have been shown in the Table (24) and the photograph in the (Plate 21B).

All the next results also followed the same procedure of calculations.

Amino acids in *Psyllobora bisoctonotata* (Mulsant) (Table 25, plate 21C)

A total number of 11 spots were appeared from the analysate which indicated the 09 amino acids as 2 were common in both the hydrolysate. Mean Rf values of the 7 spots in acid hydrolysate 0.48, 0.31, 0.39, 0.62, 0.64, 0.57were nearest to the Rf values of the standard amino acids alanine (0.48), aspargine (0.30), aspartic acid (0.40), methionine (0.60), phenyl-alanine (0.65) and valine (0.58). Similarly those of the 5 spots in alkaline solution were 0.49, 0.33, 0.35, 0.60, 0.45 resembled with the Rf values of the standard amino acids alanine(0.48), glycine (0.34), lysine (0.35), methionine (0.60) and proline (0.45). As a result 9 amino acids- alanine , aspargine, aspartic acid, glycine, lysine, methionine, proline, phenyl-alanine & proline were found in the described species which have been shown in the table (25) and photograph is in the plate- 21C.

Table 25 Rf values of the compounds present in the *Psyllobora bisoctonotata* (Mulsant) with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.48	0.48	0.49	++, ++	Alaniine
Arginine	0.22				
Aspargine	0.30	0.31		+	Aspargine
Aspartic acid	0.40	0.39		++	aspartic acid
Cystein	0.19				
Glycine	0.34		0.33	++	glycine
Glutamic acid	0.55				
Glutamin	0.15				
Histidine	0.20				
Iso-leucine	0.57	0.57	0.60	++ , ++	Iso-leucine
Leucine	0.64			+	
Lysine	0.35		0.33	+	lysin
Methionine	0..60	0.62		++	Methionine
Prolin	0.45		0.45		proline
Phenyl-alanine	0.65	0·64		+	Phenyl-alanine
Serine	0.22				
Threonine	0.43				
Tryptophan	0.70				
Tyrosine	0.38				
Valine	0.58		0.58		valine

Amino acids in *Cheilomenes sexmaculata* (Fabricius) (Table 26, plate 21)

In this species also the 9 amino acids ; alanine, cystein, glysine, glutamic acid, isoleucine, leucine, lysine, proline, tyrosine were found by the compare with Rf values of ninhydrin or iodine reacting compounds of the mean Rf values of the three spots observed from the experimental sample with those of the standard amino acids alanine, glutamic acid and lysine were common in both the acid and alkaline hydrolysate. The result was inferred on the values of 6 spots in acid hydrolysate and other 6 spots in alkaline hydrolysate. Details have been shown in the table 26 and photographs in the plate 21D.

Amino acids in *Coccinella septempunctata* Linnaeus (Table 27, plate 21)

The mean values of total number of 12spots appeared on the unidimensional TLC plate of which six were in the acid hydrolysis and another six were for the alkaline hydrolysis. A list of the chromatograms have been shown in the table . 27. The photograph of a developed plate was shown in the plate 21E.Rf values of histidine 0.24, aspartic acid 0.40, proline 0.58, glutamic acid 0.91,leucine 0.25, glysine 0.35,.alanine 0.75, phenyl alanine 0.55, lysine 0.75 , valine 0.87 and tyrosine 0.80 corresponds with the Rf values of unknown compounds 0.23, 0.4, 0.58, ,0.75 , 0.86, and 0.9 of acid hydrolysis and also correspond with the Rf values of akaline hydrolysate 0.25, 0.33, 0.52, 0.77 0.8, and 0.88 respectively which indicates the presence ofhistidine, aspartic acid, proline, glutamic acid, leucine, alanine, glysine, phenyl alanine, lysine, valine atyrosine in *C. septempunctata* Linn.

Amino acids in *Coccinella tranasversalis* Fabricius (Table 28, plate 22)

During the treatment of acid hydrolysate, the mean Rf values calculated from the spots appeared on the TLC plate were 0.18, 0.35, 0.39, , 0.45, 0.48, 0.57 & 0.76

PLATE -21

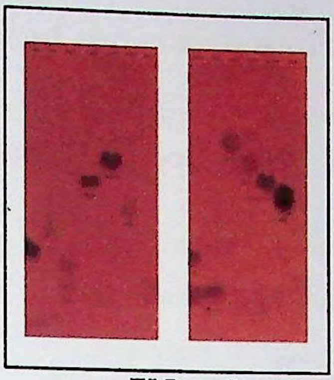


FIG - A

Developed Spots of known amino acids

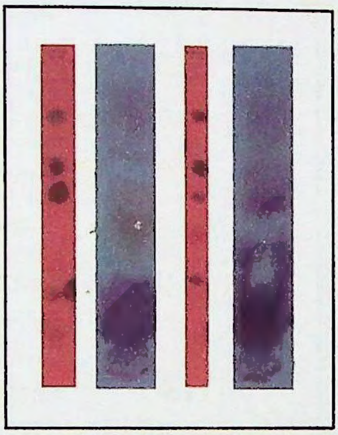


FIG - B

Developed Spots of unknown samples of
Illeis indica Timberlake

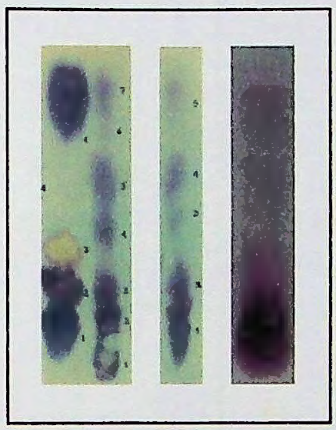


FIG - C

Developed Spots of unknown samples of
Psyllobora bisoctonotata (Mulsant)

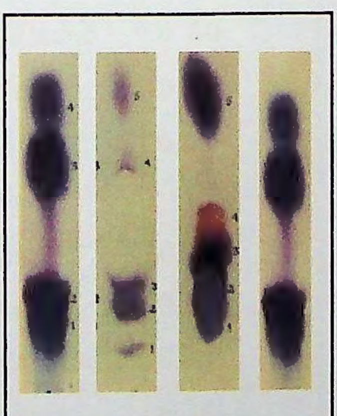


FIG - D

Developed Spots of unknown samples of
Cheilomenes sexmaculata (Fabricius)

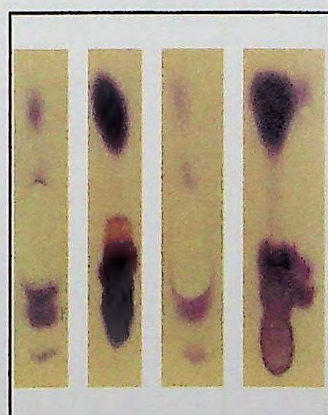


FIG - E

Developed Spots of unknown samples of
Coccinella septempunctata Linnaeus

Table 26 Rf values of the compounds present *Cheilomenes sexmaculata*(Fabricius) with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.45	0.45	0.44	++, ++	alanine
Arginine	0.25				
Aspargine	0.33				
Aspartic acid	0.40				
Cystein	0.19	0.20		+	cystein
Glycin	0.30		0.30	++	glycine
Glutamic acid	0.55	0.55		++	glutamic acid
Glutamin	0.17				
Histidine	0.25				
Iso-leucine	0.50	0.50		+	iso leucine
Leucine	0.62	0.60		++	
Lysine	0.35	0.35		++	
Methionine	0..60				
Prolin	0.40		0.40		leucine
Phenyl-alanine	0.65			++	lysine
Serine	0.23				proline
Threonine	0.44				
Tryptophan	0.66			+	
Tyrosine	0.38		0.37		
Valine	0.57				tyrosine

Table 27 Rf values of the compounds present *Coccinella septempunctata* Linnaeus with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acids detected
Alanine	0.75		0.77	++	alanine
Arginine	0.51				
Aspargine	0.30				
Aspartic acid	0.39	0.4		++	aspartic
Cystein	0.14				acid
Glycin	0.35		033	++	glycine
Glutamicacid	0.91	0.9		++	glutamic
Glutamin	0.12				acid
Histidine	0.24	0.23	0.25	+, +	histidine
Iso-leucine	0.12				
Leucine	0.45	0.44		+	leucine
Lysine	0.75	0.75		++	lysine
Methionine	0..31				
Prolin	0.58	0.58		+	proline
Phelyn-	0.55		0.52	++	
alanine					phenyl-
Serine	0.13				alanine
Threonine	0.35				
Tryptophan	0.20		0.8	+	tyrpsine
Tyrosine	0.80	0.86	0.88	++, +	valine
Valine	0.87				

similar to the Rf values of standard amino acids lysine (0.18), aspartic acid (0.35), arginine (0.40), alanine (0.45), proline (0.48), tyrosine (0.59) & phenylalanine (0.77) respectively. On the otherhand another five spots appeared on the plate, mean Rf values of which were 0.33, 0.45, 0.52, 0.63 & 0.72 showed the value nearer to Rf values of standard amino acids glysine (0.34), alanine (0.45), glutamic acid (0.50), valine (0.65) & leucine (0.73) respectively. So, the presence of the total eleven amino acids lysine, aspartic acid, arginine, alanine, proline, tyrosine, phenylalanine, glysine, glutamic acid, valine and leucine can be inferred in this species. The details have been shown in the table. 28 and photograph is in the Plate 22 A.

Amino acids in *Harmonia octomaculata* (Fabricus) (Table 29, plate 22)

Harmonia octomaculata (Fabricus), the species under the same tribe coccinellinii of the above described two species showed the presence of 10 amino acids alanine, aspartic acid, glycine, glutamic acid, histidine, lysine, proline, phenyl-alanine, threonine and tryptophan , Rf values of which were 0.52, 0.53, 0.58, 0.25 ,0.47, 0.50, 0.55, 0.52, 0.35 & 0.38 correspond with the mean Rf values of 8 compounds in acid hydrolysate – 0.50, 0.35, 0.57, 0.25, 0.47, 0.50, 0.55 and 0.51 as well as the 6 compounds in the alkaline ones – 0.52, 0.34, 0.23, 0.52, 0.35 & 0.38 which have been shown in the table 29 and the photograph is in the plate 22 B.

Amino acids in *Micraspis discolor* (Fabricius)(Table 30, plate 22)

Under the same tribe coccinellinii, in this species also 10 amino acids found compare with the Rf values of spots appeared on the TLC plate in the standard amino acids. Mean Rf values of 2 spots in each sample were common. Rf values of the unknown compounds showed the similarities to the Rf values of the standard amino acids alanine, aspartic acid, glycine, glutamic acid, histidine, lysine, proline, threonine, tyrosine & valine. So their presence in this species can be inferred. List of the chromatograms in the table. 30 related photograph plate 22 C.

Table 28 Rf values of the compounds present in the *Coccinella transversalis* Fabricius with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acids detected
Alanine	0.45	0.45	0.45	++	alanine
Arginine	0.40	0.39		+	arginine
Aspargine	0.37				
Aspartic acid	0.35	0.35	0.34	++, +	aspartic
Cystein	0.09				acid
Glycin	0.30		030	++	glycine
Glutamic acid	0.50	50	0.52	++, ++	glutamic
Glutamin	0.15				acid
Histidine	0.26				
Iso-leucine	0.79				isoleucine
Leucine	0.73				
Lysine	0.20	0.20		+	lysine
Methionine	0..51				
Prolin	0.48	0.46		++	proline
Phelyn-alanine	0.75		0.75	++	phenyl-alanine
Serine	0.23				
Threonine	0.45				
Tryptophan	0.30				
Tyrosine	0.59	0.58		++	tyrosine
Valine	0.65		0.63	+	valine

Table 29 Rf values of the compounds present in *Harmonia octomaculata (*Fabricus) with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.52	0.50	0.52	++	alanine
Arginine	0.33				
Aspargine	0.41				
Aspartic acid	0.35	0,35	0.34	++	aspartic acid
Cystein	0.19			++	glycine
Glycin	0.58	0.57			
Glutamic acid	0.25	0.25	0.23	+	glutamic
Glutamin	0.20				acid
Histidine	0.47	0.47		+	histidine
Iso-leucine	0.64				
Leucine	0.60				
Lysine	0..50	0.50		++	
Methionine	0.45				lysine
Prolin	0.55	0.55		++	proline
Phenyl-alanine	0.52	0.51	0.52	++ ,++	Phenyl-
Serine	0.40				alanine
Threonine	0.35		0.35	+	threonine
Tryptophan	0.38		0.38	+	tryptophan
Tyrosine	0.58				
Valine	0.18				

Amino acids in *Micraspis yasumatsui* Sasaji (Table 31, plate 22)

When the acid hydrolysate was co-chromatographed with standard amino acids the mean Rf values calculated were 0.42, 0.39, 0.35, 0.49, 0.55, 0.69, 0.25 similar to the Rf values of the amino acids alanine (0.43), aspartic acid (0.40), glycine (0.34), glutamic acid (0.50), lysine (0.55),phenyl-alanine (0.69) & serine (0.25) . Another 6 spots were appeared in the alkaline hydrolysate , Rf values of which were 0.29, 0.40, 0.33, 0.50, 0.53, 0.45 correspond with the Rf values of some standard amino acids arginine (0.29), aspartic acid (0.40), glycine (0.34), glutamic acid (0.50), lysine (0.55) & proline (0.45) respectively. Of the total numbers of values 4 values in each hydrolysate indicated the same 4 amino acids . So the 9 amino acids – alanine, arginine, aspartic acid, glycine, glutamic acid, lysine, proline, phenyle-alanine and serine can be inffered as the amino acids present in *Micraspis yasumatsui* Sasaji Details result have been shown in the table 31 and the photograph is in the plate 22D

Amino acids in *Afidenta misera* Mulsant) (Table 32, plate 23)

This species under the sub family Epilachninae showed the presence of 9 amino acids alanine, arginine, glycine, leucine, lysine, proline, phenyl-alanine, serine & valine ; Rf values of which 0.53, 0.38, 0.22, 0.68, 0.40, 0.46, 0.77, 0.32, 0.65 were correspond with the mean Rf values 0.53, 0.37, 0.40, 0.45, 0.77, 0.30, 0.65 in acid hydrolysate and those of the o.52, 0.38, 0.22, 0.68, & 0.75 in alkaline hydrolysate. A list of the chromatograms have been shown in the table (32) and photograph in the plate (23A)

Amino acids in *Epilachna septima* Dieke (Table 32, plate 22)

A total number of 11 spots of ninhydrin as well as iodine reacting compounds in both acid and alkaline samples showed the presence of 8 amino acids as three spots in each

Table 30 Rf values of the compounds present in the ladybird beetle*Micraspis discolor* (Fabricius) with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acids detected
Alanine	0.45	0.45	0.45	++	alanine
Arginine	0.61				
Aspargine	0.37				
Aspartic acid	0.35	0.35	0.33	++	aspartic
Cystein	0.29				acid
Glycin	0.34			++	glycine
Glutamic acid	0.40	0.41	0.40		
Glutamin	0.15			++	glutamic
Histidine	0.27				acid
Iso-leucine	0.69		0.25	+	histidine
Leucine	0.73				lysine
Lysine	0.20				proline
Methionine	0..61		0.19	++	
Prolin	0.48	0.47			threonine
Phenyl-alanine	0.77			+	
Serine	0.23				
Threonine	0.44	0.44		+	
Tryptophan	0.80				
Tyrosine	0.59	0.60		++	tyrosine
Valine	0.75		0.76	+	valine

PLATE -22

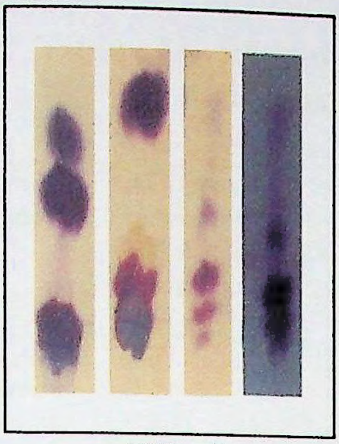


FIG -A

Developed Spots of unknown samples of *Coccinella transversalis* Fabricius

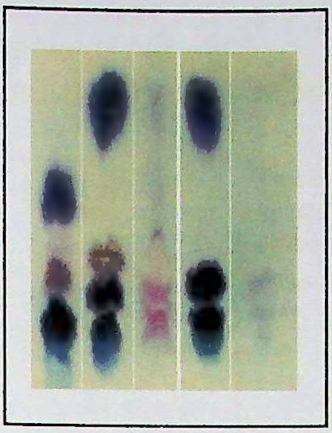


FIG - B

Developed Spots of unknown samples of *Harmonia octomaculata* (Fabricius)

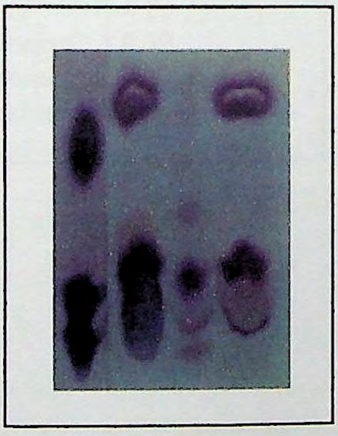


FIG -C

Developed Spots of unknown samples of *Micraspis discolor* (Fabricius)

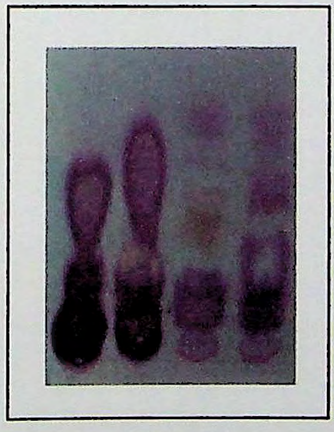


FIG -D

Developed Spots of unknown samples of *Micraspis yasumatsui* Sasaji

Table 31 Rf values of the compounds present in *Micraspis yasumatsui* sasaji with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysate	Rf values of compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acids detected
Alanine	0.43	0.42		++	alanine
Arginine	0.29		0.29	+	arginine
Aspargine	0.20				
Aspartic acid	0.40	0.39	0.40	++,++	aspartic acid
Cystein	0.15				
Glycin	0.34		0.33	++	glycine
Glutamic acid	0.50	0.49	0.50	++	glutamic acid
Glutamin	0.21				
Histidine	0.57				
Iso-leucine	0.65				
Leucine	0.60				
Lysine	0.55	0.55		++	lysine
Methionine	0.67				
Prolin	0.45		0.45	+	prolin
Phenyl-alanine	0.70	0.69		++	phenyl-alanine
Serine	0.25	0.25		+	serine
Threonine	0.33				
Tryptophan	0.38				
Tyrosine	0.58				
Valine	0.64				

media indicated thecommon values. Amino acids alanine, arginine, glycine, leucine, lysine, phenyl-alanine, serine and valine were found in the present species of the Epilachninae . Details result havebeen shown in the table 33 and photograph in the plate 23B.

Amino acids in *Epilachna pusillanima* Mulsant) (Table 34, plate 23)

Eight spots in acidic extract and seven in alkaline ones of this species under the above described sub family indicated the presence of total 10 amino acids alanine, arginine, glycine, ,leucine, lysine, methionine, proline, phenyl-alanine, serine & tryptophan; Rf values of which were resembled with those of the unknown compounds. Table 34 shows the total list of chromatograms where Rf values of alanine (0.55), lysine (0.25), proline (0.50), serine(0.70) & tryptophan (0.73) resemble the Rf values of unknown compounds in both of acidic and alkaline media. Photograph have in the plate. (23C)

Amino acids in *Epilachna vigintioctopunctata* Fabricius) (Table 35, plate 23)

Same numbers amino acids like above ones found in this species of the same sub family Epilachninae. In acid hydrolysate seven spots developed on the TLC plate, Rf values of which 0.48, 0.35, 0.62, 0.70, 0.64, 0.20 were resembled with the Rf values of standard amino acids alanine (0.48), glycine (0.36),), leucine (0.62), methionine (0.70), phenyl-alanine (0.65) and serine (0.22) respectively. Another six spots were Another six spots were appeared in alkaline hydrlysate , Rf values of which were 0.49, 0.55, 0.33, o.69, 0.45, 0.60 similarly resembled the standard amino acids alanine, arginine (0.55), lysine (0.35), methionine, proline(0.45) and tryptophan (0.60) respectively. A list of the chromatograms have been shown in the table (35) & photograph in the plate (23D).

Table 32 Rf values of the compounds present in *Afidenta misera* Mulsant with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Amino acid detected
Alanine	0.53	0.53	0.52	++	alanine
Arginine	0.38	0.37	0.38	+	arginine
Aspargine	0.25				
Aspartic acid	0.34				
Cystein	0.20				
Glycin	0.22		0.22	++	glycine
Glutamic acid	0.49				
Glutamin	0.15				
Histidine	0.26				
Iso-leucine	0.70				
Leucine	0.68		0.68	+	leucine
Lysine	0.40	0.40		++	lysine
Methionine	0..60				
Prolin	0.46	0.45			proline
Phenyl-alanine	0.77	0.77	0.75	+ ++	Phenyl-alanine
Serine	0.32	0.30		+	serine
Threonine	0.43				
Tryptophan	0.70				
Tyrosine	0.58			+	valine
Valine	0.65	0.65			

Table 33 Rf values of the compounds present in *Epilachna septima* Dieke with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.53	0.52	0.51	++	alanine
Arginine	0.38		0.38	+	arginine
Aspargine	0.15				
Aspartic acid	0.35				
Cystein	0.11				
Glycin	0.24	0.25	0.23	++	glycine
Glutamic acid	0.40			+	
gulamin	0.28				
Histidine	0.28	0.75			
Iso-leucine	0.73	0.35			
Leucine	0.74			++	leucine
Lysine	0.35				lysine
Methionine	0..60	0.78			
Prolin	0.48			++	Phenyl-
Phenyl-alanine	0.77			++	alanine
Serine	0.34		0.33		serine
Threonine	0.43				
Tryptophan	0.66	0.60			
Tyrosine	0.50				
Valine	0.62		0.61	+	valine

Table 34 Rf values of the compounds present in *Epilachna pusillanima* Mulsant with standard amino acids

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.55	0.55	0.56	++	alanine
Arginine	0.25			+	arginine
Aspargine	0.33				
Aspartic acid	0.45				
Cystein	0.18				
Glycin	0.49		0.48	++	glycine
Glutamic acid	0.60				
Glutamin	0.32				
Histidine	0.38	0.57			
Iso-leucine	0.58	0.65	0.23	++	Iso-leucine
Leucine	0.65	0.70		+	Leucine
Lysine	0.25	0.25	0.54	+	lysine
Methionine	0.55	0.73	0.51	++	Methionine
Prolin	0.50	0.50		++	proline
Phenyl-alanine	0.63	0.62		++	Phenyl-alanine
Serine	0.70		0.69	++	serine
Threonine	0.23			+	
Tryptophan	0.73		0.72	+	Tryptophan
Tyrosine	0.40				
Valine	0.35				

Table 35 **Rf values of the compounds present in *Epilachna vigintioctopunctata* Fabricus with standard amino acids**

Standard aminoacids	Rf values obtained in standard amino acids	Rf values of unknown compounds in acid hydrolysaye	Rf values of unknown compounds in alkaline hydrolysate	Relative intensities of the colour produced	Aminoacids detected
Alanine	0.48	0.48	0.49	++, ++	alanine
Arginine	0.55		0.55	+	arginine
Aspargine	0.30				
Aspartic acid	0.40				
Cystein	0.22	0.35	0.33	++	glycine
Glycin	0.16				
Glutamic acid	0.36				
Glutamin	0.19				
Histidine	0.21				
Iso-leucine	0.27			+, +	leucine
Leucine	0.62	0.62	0.69		
Lysine	0.87		0.45	++	lysine
Methionine	0.62	0.70		++	methionine
Prolin	0.45			+	proline
Phenyl-alanine	0.70	0.64		++	Phenyl-
Serine	0.22	0.20			alanine
Threonine	0.65			++	serine
Tryptophan	0.60		0.60	+	tryptophan
Tyrosine	0.22				
Valine	0.38				

PLATE -23

FIG -A

Developed Spots of unknown samples of *Afidenta misera* Mulsant

FIG - B

Developed Spots of unknown samples of *Epilachna septima* Dieke

FIG -C

Developed Spots of unknown samples of *Epilachna pusillanima* Mulsant

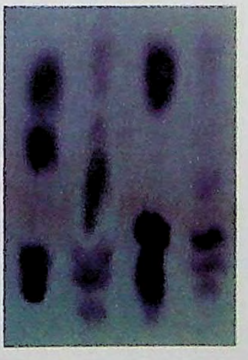


FIG -D

Developed Spots of unknown samples of *Epilachna vigintioctopunctata* Fabricius

CHAPTER - 5

DISCUSSION

Discussion

In 1931 Lewitsky published two important papers, one dealing with karyo systesmatics and the other, as was termed as idiograms to represent the length, centromeric positions and secondary constrictions of the chromomosomes in a wide range of species. At that time idiogranm had become an important datum for chromosomal studies. Later the idiogram idea was extended to the karyotype concept. Karyotype is still taken to indicate a systematic array of chromosomes of mitotic or meiotic cell involving number, form, size, and other features that may typify the cell complement of an individual or species. Quantitative studies in karyotypic analysis involving number, structure and hydrochemistry have illuminated spectacular genetically analysis of the species concerned (White, 1954; Lewis and John, 1963) Further developments concerning the enzyme polymorphism of Coccinellidae by Sasaji & Ohnishi (19730a, 1973b), Sasaji (1974),Tanimoto (1975) and Kuboki (1978). Amino acids are the main components of enzymes as well as the alphabet of genetics (Sharma 1976). Genetic differentiation during speciation have been discussed by Ayala *et al.* (1974).

The characteristic intra specific polymorphism of color peculiar to many species of Coccinellidae has long attracted this attention of population geneticists The correlation between the phenotypic color polymorphism and chromosomal polymorphism in this family may be shown primarily with the advancement in technique for analysis the chromosomes and secondly with the accumulation of sufficient comparative karyological data.

Chromosomal polymorphism.

Structural variations in chromosomes are the main causes of remarkable polymorphism met in the various group of animals. Chromosome changes involving many different kinds have naturally occurred in related species. Some of these

Table 36 Chromosomal data of several reported species of Coccinellidae

species	male (2n)	chromosome formula(2n)	References	Present Investigation male (2n)
Epilachninae				
Epilachna vigintioctopunctat (F)	18	8AA+XY	Yosida 1948, Bose 1948.	18
		8AA+XY	Agarwal 1960-1961	(8AA+Xy)
		$8AA+XY_p$	Yadav *et el.*, 1979	
E. orientalis Zimm.	18	8AA+XY:XX	Agarwal 1960, 1961	
E. Dodecastigma Wied.	20	$8AA+XY_p$	Lahiri & Manna 1969	16
(=E.Pussillanima)	12 & 14	5AA+XY, $6AA+X_rY_r$	Saha & Manna 1971	(7AA+Xy)
E. septima Dieke	20	$9AA+Xy_p$	Kacker 1973	20
				(9AA+Xy)
Coccinellinae Scymnini				
Scymnus nubilis Muls.	-	7AA+neo-XY	Smith 1960b	14
				(6AA+Xy)
Pharascymnus sp.	-	9AA+ Xyp	Smith 1960b	20
				(9AA+Xy)
Chilocorini				
Chilocorus nigritus (F)	22	10AA+Neo-XY	Yadav & pillai 1974	
			Yadav *et. el.*, 1979	
C. circumdatus	-	10AA+neo-XY	Takenouchi 1976	
C. Hauseti Wse	-	10AA+neo-XY	Takenouchi 1976	
Brumaus suturalis (F)	18	8AA+neo-XY	Yadav & pillai 1974	
			Yadav *et. el.*, 1979	
Exochomus lituratus 1 Gorham	18	8AA+XY	Smith 1965a	
E. lituratus 2 Gorham	18	8AA+XY	Smith 1965a	
E. uropygialis 1 Muls.	16	7AA+XY	Smith 1965a	
E. uropygialis 2 Muls.	16	7AA+XY	Smith 1965a	
E. uropygialis 3 Muls.	18	8AA+XY	Smith 1965a	
Synonichini				
Menochilus sexmaculatue (F)	20	9AA+Xyp	Agarwal 1960, 1961	20
(=Chillomenes sexmaculata)			Yadav *et. el.*, 1979	(9AA+Xy)
Micraspis cardoni (Weise)	20	9AA+Xyp	Yadav & pillai 1974	
			Yadav *et. el.*, 1979	
Verania discolor (F)	20	9AA+Xyp	Manna & Lahiri 1972	
V. allardi Muls.	20	9AA+Xyp	Yadav & pillai 1974	
			Yadav *et. el.*, 1979	
Coccinellini				
Coccinella reponda Thumb.	20	9AA+Xyp	Agarwal 1960, 1961	
C. transversalis (F)	20	9AA+Xyp	Manna & Lahiri 1972	20
			Dua & Kacker 1975	(9AA+Xy)
C. septimpunctata L.	20	9AA+Xyp	Sharma *et. el* 1959	20
			Agarwal 1960, 1961	(9AA+Xy)
			Manna & Lahiri 1972	
Summus cardoni Weise	18	8AA+Xyp	Manna & Lahiri 1972	
Afissa parvula (Cr.)	18	8AA+Xyp	Kacker 1976	
Psylloborini	20	9AA+Xyp	Yadav & pillai 1974	20
Illeis indica Timbeilae			Dua & Kacker 1975	(9AA+Xy)
Thea biscoctonata Muls	20	9AA+Xyp	Yadav & pillai 1974	
Noviini	-	8AA+X	Smith unpublish	
Rodolia cardinalis Muls				
Harmonia axyridis spectabilis	-	$7AA+Xy_p$	Smith unpublish	
Asalia bipunctata L. (Wash.)	20	$9AA+Xy_p$	Smith unpublish	

changes have been important in elucidating cytogenesis principles concerning chromosome behavior. They many include the changes in number, size, shape, centromeric position, primary and secondary constrictions, hetero-chromatin and euchromatin, satelite as well as some important chromosomal aberration including translocations, inversions, duplications. The crucial observation of the constancy of the chromosome number for a particular species was promptly set aside by some subsequent splendid discoveries in plant species when Guignard (1891) found different chromosome numbers in different genera of Liliaceae. Following this discovery in numerous studies with regard to chromosome numbers, it was obvious that species of the same genus might have different chromosome number.

The changes in the chromosome number bears a direct relationship to the genetic evolutionary process than do any other types of changes. The commonest type of changes in the chromosome number appear to be the polyploidy. This is a type of irreversible change, where primitive forms bear the lowest number of chromosomes. But in animal cells polyploidy is rare (Bugenberg, 1957; Suamalainen, 1958). The relative scarcity of polyploidy in the case of animals in sharp contrast to higher plants, might be due to the relatively sensitive balanced chromosomal mechanism between the sexes. Any imbalance in this chromosomal mechanism might result in inter sex or other types of disturbances. Rather changes in chromosome number may be seen to involve an increase or decrease by one chromosome at a time. This aneuploid alteration of basic number by loss or gain of a chromosome may be produced by unequal translocation (Darlington, 1937). Evidence in majority cases are conclusive that aneuploid alteration of basic number of chromosomes results in higher basic number in the most primitive species

Monosomics resulting in loss, appears to have more deleterious genetic change, however has no t been a common place in coccinellids .

Variable chromosome numbers without apparent gain or loss of critical genetic materials have been found to be associated with the centromere in the form of centric fusion or centric fission. Both these processes of fusion and fission may result in increase or decrease of chromosomal number without, however, affecting the number of major arms of chromatid (Matthey,1958). Despite an extensive work on coccinellids chromosmes, their exact morphological characterization is still inadequate. The findings of various sources reveal great many contradictions in the question of number and morphology of the karyotype. The typical coleopteran diploid chromosome as established by Yosida (1952), Smith (1960) consists of 20. Moreover the diploid number of the family coccinellidae as ranges from 12 to 26 was stated by Kacker (1993).

But in the sub family Coccinellinae, which includes mostly entomophagous beetles, the situation is very heterogenous. The diploid number varies from 12 to 24 (Kacker (1993).

The sub family Epilachninae is more or less homogeneous and has the typical coleopteran number 2n=20. The chromosomes of two species of Epilachninae *Afissa parvula* and *Epilachna septima* were described by kacker (1993) of which the former had 2n=18 and the latter 2n=20.

Afissa parvula besides having one fewer autosomal pair had a very large X chromosome. Reduction in diploid number in the Epilachninae had also been noticed in the hybrid between *E. chrysomelina* and *E capensis* (strasburagar, 1936) and in *E vigintioctopunctata (=Henosepilachna vigintioctopunctata)*. (Yosida 1944, Bose 1948 and Agarwal 1961). The reduction in number could be due to autosome (A-A) fusion and enlargement of the X chromosome. In *Afissa parvula* to X –A fusion reported by kacker (1993). In the tribe Hyperspini Smith and virkki (1978) have studied some species of *Hyperaspis* of which all but two had 2n=7.

The large number of variation in the diploid number of chromosomes of *Epilachna dodecastigma* from 12 (5 AA + Xy) to 25 (11+XXy) in the males and 26 (11+XXXX) in the females (Saha and Manna, 1971). Yadav *et al.* (1979) studied the chromosome of six species of Coccinellidae from Haryan (India), there it was found in *Epilachna vigintionctopunctata* 2n=20 where 9 pairs of autosomes and the sex chromosomes X and y.

The present findings showed 2n=18 chromosomes in each spermatogonial cell of *Epilachna vigintionctopunctata* in the form of 8AA+Xy which agreed with the findings of Agarwal (1961), 2n=18 in *Epilachna vigintionctopunctata.*

Mittal (1989) reported 2n=18 chromosomes in *Epilachna indica.*

Epilachninae have the chromosomes 2n= 10, 18 and 20 in most of the species, except *Epilachna dodecastigma* (=*Epilachna pusillanima*) where 2n=14 (Saha, 1973) . In that point present report on *Epilachna vigintionctopunctata* coincides the report of Saha (1973). Further more reports were available that , all the series belonging to the sub family Epilachninae, so far have either 18 or 20 chromosomes and an Xy : XX mode of sex determination (Stevens 1906, 1909, Hoy 1918, Strasburger 1936, Yosida 1944, 1948, Takenouchi 1955). This reports also support the present report on Epilachninae.

Karyotypic differentiation in the *E. vigintioctomaculata* complex and its possible relevance to the reproductive isolation studied by Tsurusaki *et.al.* (1993). They revealed 2n=20 in all the species of the complex except for that of some males of *E pustulosa* (2n=21) with a supermumery Y chromosome, but they successfully revealed an apparent differentiation in Karyotype between *E vigintioctomuculata* complex and three other species *E pustulosa, E niponica* and *E yasutomii.* It was shown that the major difference comes from addition of long hetenochromatic segments in Nos 3-9 chromosomes in the later three species. Three populations of *E . Vigintioctomaculata* representing three different geographic forms Hokkaido, Honshu and Rishiri were differed in both relative length and arm ratio. The Hokkaido form with 2n=20, consisted a pair of large metacentric and 8 (Nos. 2-9) pairs of submetacentric autosomes In the other two forms too, autosomes were similar except for 2 pairs of sub telocentrices (Nos 3-7) in Hanshu form and a pair of metacentrics (No.2) in Rishiri. The X chromosome, largest and metacentric in the former two (Tsurusaki *et al.*,1993). The spermatogonial complenent of *E vigintioctopunctata* (Fab) investigated by authoress observed to have 18 chromosomes (16 A+Xy) of which 6 pairs of autosomes, metacentric and Nos. 4 and 5 are sub metacentric. The largest X chromosome is also metacentric, y is the smallest chromosome. In the present investigation another two species of Epilachna ie. *E. Septima* Mulsant. and *E Pusillanima (*Muls*)* showed 2n=20 and 16 respectively In *E. septima* Nos 1,2,4 and 5 pairs chromosomes were submetacentric. , Nos. 3 was telocentric and others including X chromosome was metacentric. In *E pusillanima* of the autosomes Nos 1-4 and 7 were sub metacentric and Nos 5 and 6 were metacentric. X was also metacentric. y chromosome in each species was the smallest. In the present investigation another species of the sub family Epilachninae *Afidenta misera* Mulsant showed 2n=14 (6AA+X+y); of the autosomes Nos. 3,4,6 were metacentric, Nos. 2 and 5 were submetauntric and Nos. 1 was telocentric X chromosome was sub metacentric, y was also with undistinguished type of centromere. The chromosome complement of this species are unlike other Epilachninae. No previous report on chromosome of this species is available. Although Saha (1973) reported 2n=14 in *Epilachna dodecastigma* (=*E.pusillanima*) of the Epilachninae. In the present study 2n=16 in *E pusillanima,* the same number of chromosomes were reported in another species *Afissa purvulosa* of the Epilachninae (Kacker ,1993). Shiwago (1931) reported X chromosome as the largest and easily recognizable while y is always smallest Yosida (1948) also observed the presence of y as a minute chromosome. The present findings of the four species of Epilachninae showed the chromosome number and sex determining mechanism centering round the type number 2n=14 to 20 (Smith 1960).

It was previously mentioned that in the sub family Coccinellinae, the diploid number of chromosome varies from 12 to 24 in spermatogonial cell (Kacker,1993). Present investigation on the 16 species of the Coccinellinae showed 2n=14,16,18 and 20.

The spermatogonial complement of *Coccinella repanda* and *C. septempunctata,* investigated by Agarwal (1960) where it was observed that 2n=20 chromosomes (18 autosomes+X+y). Chromosome number in *C. septempunctata L.* was reported 2n=20 (9AA+Xyp) by Sharma *et. at.,* (1959) and Agarwal (1960, 1961). A list on the chromosome number of Coleoptera was published by Smith (1960) which also showed 2n= 20 chromosomes in male *C. septempunctata* . From the karyological investigations of five Indian coccinellids by Mittal *et al.* (1989) it was found that *Micraspis discolor* and *C. septempunctata* possess 20 chromosomes in each gonodial cell. Reports on another species , *C. transversalis* (Manna and Lahiri, 1972 Dua & Kacker 1976, Dasgupta , 1977) show that have 2n= 20 . The authoress has also reported 2n = 20 chromosomes in the above three described species *Micraspis discolor, Coccinella septempunctata* and *C. transversalis* (*C.repanda=C. transversalis*) . In this study, other species , *Micraspis yasumatsui* showed 20 chromosomes in the diploid cell . No record on the chromosomes of this species(*C. yasumatsui*) was available to the authoress. However, it agreed with the modal number ,reported by Smith (1960). Further more another species *Micraspis cardoni* (Weise) showed 2n=20 (Agarwal, 1961).

Numerical and morphological polymorphism of chromosome have been observed in many species of Coccinellini like *Harmonia arculata* (Fab) and *H. axyridis* showed the spermatogonial complements of 16 chromosomes (7AA+Xyp) (Simith 1962, 1965). The species *H. octomaculata* (Fab) studied by the authoress reported 2n=16 (7AA+Xy) i,e, the sex mechanism differed from *H arculata* where no parachute form was detected within the sex chromosomes.

Another Haryanan species *Menochilus sexmaculata* showed 2n = 20, where autosomes were submetacentric or acrocentric, X was acrocentric and y was spherical (Yadav *et.* 1979). In the same species it was previously reported by Agarwal (1961) 2n = 20. Present findings in *Cheilomenens sexmaculata* (=*Menochilus sexmaculata*) also agreed with Agarwal (1961), 2n = 20. Agarwal (1961) observed there only the two pairs of m chromosomes while present result showed the total complement consisted all the m types of chromosomes.

The present report also deals with a new species *Apomycraspis quayumi* near *Micraspis* found in Bangladesh. The spermatogenial cell of *which,* showed 2n=20 (9AA+Xy). As a new species no scope of previous reports but its result numerically agreed with the genus *Micraspis.* However, other cytotaxonomic and cytogenetic features do not tally with other two *Micraspis species* .

Another species of the tribe Coccinellini *Propylea quuatuordecimpunctata* Linn. also showed here, 2n = 20 (9AA + Xy) agreed the modal number 2n= 20 (Smith, 1960). No other report on it was available.

The Psylloborinii species Phyllobora *Faedata* Lec of california (Smith 1960) and *Illeis indica* Timbeilake of India (Yadav & pillai 1974, Dua and Kacker 1976) have been studied previously. Of the two investigations former one showed 2n=18 (8AA+ Xy) and the later showed 2n =20 (9AA + Xyp) with y in a form. The present report on psylloborini species of Bangladesh *Psyllobora bisoctonotata* (Musant) showed 2n=18 (8AA+Xy); of the autosome pairs Nos. 1,2,7 were metacentric and others submetacentric X chromosome was large metacentric, y was minute. Another species *Illeis indica* Timbeilake showed numercally the chromosome was typical i,e i2n=20 where autosomes were telocentric and submetacentric. X chromosome was metacentric, y was also minute which agrees with the findings of Yadav & pillai (1974), Dua and Kacker (1976).

The Synonychini and a part of the Scymnini showed, in most of th eir species, the typical 2n=20 (Li 1940; Yosida 1944, Smith 1953 a. 1960a).The only aberrant case in the Synonychini was *Aiolocaria mirabilis* which 2n=16 AA+X (Makino 1951). Some deviant Karyotypes were reported. Of the sub family Sticholotidinae, the Scymnini has followed two trends, the first one towards secondary acquisition of neo-Xy as evidenced in 4 species of *Scymnus*, which had 2n=16 of which 7 pairs were autosomes and neo-Xy, the second is towards an increase in chromosome number as seen in *Cryptolaemus montrouzieri*, which had 2n=22. The spermatogonial complement of *Scymnus nubilis* Mulsant, Investigated by the authoress was observed as 2n=14 (6AA+Xy) of which only the autosome pair No 4 was sub metacentric and all the others including X-chromosome were metacentric. y chromosome was minute. *Pharoscymnus taoi* Sasaji studied at the same time showed 2n=20 (9AA+X+y). Smith (1960b) established that spermatogonial complement of *pharoscymnus sp.* have 20 chromosomes of 9 pairs of autosomes and X chromosome associate with y chromosome in a parachute form. Both fission and fusion mechanism were presumably have involved in the evolutionary change of chromosome number. Another species of the Scymninae, *Stethorus tetranychi* kapur was also studied here where the spermatogonial cell showed 2n = 20 (8 AA + Xy). Of the Sticholotidinae,in *Jauraivia pullidula* Motschulsky 2n=18(8AA+Xy) was found in the present investigation. No previous reports on chromosomes of these two species was found.

Chilocorus stigma (Smith 1959) indicates that the group is a potential source of genetic variability which may be tapped through controlled mating and vigorous selection. Nevertheless the typical polyphagan chromosome number 20 (9 AA+Xyp) in male is quite common in Coccinellinae but absent from the Epilachninae. In *Chilocorus*, the doploid number of chromosomes varies from14 in C *hexacyclus* to 26 in the female *C. stigma (*Yadav *et. al,*1979). 2n =22 in the species *chilocorus nigritus* (F) of which autosomes were metacentric or submetauntric, X metacentric and y slightly submetacentric In *Brumus sutaralis* (F (= *Brumoidus sutaralis*) 2n =18 metacentric or sub metacentric chromosomes (Yadav *et al.* 1979*)*. Present report on

Bumoides lineatus (weise) showed 2n =18 (8 AA + Xy) alongwith metacentric, sub-metacentric and telocentric chromosomes.

Rodolia fulvescens Hoang the species of the sub family Coccidullinae, studied in present showed 2n = 20 (9AA+Xy). *Rodolia cardinalis* Muls. of France and California studied by Smith (1960) where he reported 2n = 17 (8AA+X) . There was only the X chromosome, no y chromosome was found.

The principle governing the changes involving the increase and decrease i,e, the aneuploidy alteration of the basic chromosome number is now well established in cytological literatures. The reasons underlying the basic number of chromosomes have been explained by various workers. The main factor attributed are unequal translocation, centric fusion and centric fission etc. On the other hand the phylogenetic increase in chromosome number may came out as a result of differences in the number of telocentric and metacentric chromosomes arms (Robertson, 1916). When metacentric chromosome arises by fusion of two telocentric chromosome a phenomenon known as Robertsonian fusion.

However, it must be borne in mind that relative fragility of the hypotonically treated metaphase cells, conventional techniques may lead to the loss of chromosomes during preparation of microscope slides. Thus great care and precision was taken to avoid this or to eliminate from analysis cells which are obviously disrupted.

Chromosome shape and size

The shape and size of the chromosomes seem to be of great value in the cytotaxonomy and karyotypic evaluation. The coccinellide chromosome is not worthy for having no extra large chromosome and majority show a smooth transition of size from the largest to smallest. It is difficult to make a systematic classification of the chromosome based on the size an deposition of the centromere (Robinson, 1972), The remarkable continuity in the gradation in size of the chromosomes was depicted in the idiogram prepared by Levan *et al.*, (1962). However, attempts have been made to class the autosome (Slizynsky, 1955) as long, medium and short. Tjio and Levan (1953) grouped them in four like that of Goldjman *et at* (1966). All these classifications, however, seriously suffered from realistic approach.

A recent work by Mittal *et. at* (1989) exhibited the Karyotype of five Indian coccinellids of which *Epilachna indica* Mulsant showed all the autosomes ranging from 4.04μm to 2.26μm Of the sex chromosomes, the X was the largest in the complement being 6.40μm while y was measured to be 1.60μm and the total length of the haploid set of was found to be 31.47μm .

Kacker (1993) explored the morphometric data of *Afissa parvula* (Crotch) and obtained mean relative length in percent 13.7 to 7.1 in autosomes and 19.4 in X chromosome while y was only 2.1. Tsurusaki *et. at.*, (1993) studied karyotypes of *Epilachna vigintioctomaculata* complex. The complex showed the percentage of total chromosomal length (TCL) Longest pair of autosome ratio ranges from 13.4 to 10.4 and shortest pair ranges from 5.9 to 5.2. The X chromosomes from 15.2 to 10.4 and y chromosome from 4.00 to 1.2. The chromosomes of the two beetles of complex were with all metacentrics autosomes; in the others both meta and submetacentric types of autosoms but all the X chromosomes were sub-metacentrics. Present findings could be reported that most of the chromosomes of the Epilachninae are metacentric type and the highest ratio of RL comprises the X chromosome (18.80 %) of *Epilachna vigintioctopunctat* Agarwal reported *Epilachna vigintioctopunctata* Consisted of eight pains of oval curved rod-shaped chromosomes and a pair of metacentric ones. The metacentric chromosomes were not identical but vary in their shaped and size. After the morphological analysis, it became evident that the largest 'V'-Shaped element was the X-Chromosome and the other metacentric one was the Y. In his study *Epilachna Orientalis* showed all the chromosomes were rod-shaped acrocentric in nature except the two large metacentric. while the present study on *Epilachna vigintioctopunctata* has shown two pairs of ' J' shaped autosomes .

In another investigation the total length of the haploid set of chromosomes of *Coccinella septempunctata* Linn was found to be 35. 65µm ranging from 5.41µm to 1.77µm in autosomes (Mittal *et. at* 1989) according to whose observation the length of X and y were 5.95µm and o.98µm respectively. The authoress reported that in *C. septempunctata* Linn total length of the genome was 42.00µm and the RL of AA varied from 05.44 % to 12.49 %.

A general characterization involving brilliant chromosomal preparation and showing finner details may have recorded more positive recognition which was, however, not possible under the context, the research was undertaken. Nevertheless, much of the fundamental anomalies could be averted following Leven *et. al.*, (1962). These workers pinpointed the largest and the smallest of the autosomes. The secondary constriction in some of the other chromosomes may be of considerable use in identification. As has been mentioned earlier Ford and Woollam (1963) identified the largest and the smallest chromosome. These two also used secondary constriction as additional systematic values of identification of the chromosomes. Bennet (1965) suggested some factors as length, degree of contraction, angle of arms and morphological differentiation of centromere region could be of some value in this regard.

According to Agarwal (1961) testicular cells of *Coccinella repanda* consisted of one pair of 'J' shaped two pains of 'V' shaped and six pains of rod-shapes autosomes; in *C. septempunctata* 14 'V' or 'J' shaped metacentric chromosomes, 5 rod shaped, acrocentric ones and a minute spherical body represented y chromosome, X was acrocentric. The authoress reported 'V' and 'J' shaped chromosomes in AA at No 5 and No 8 respectively while the X was also acrocentric like the findings of Agarwal (1961). Another species of *C. transversalis* exhibited most of the metacentric type of AA.with rod shaped X chromosome.

This variation in morphology of chromosomes regarding the meta, submeta, subtelo and acrocentric nature, results in variation from species to species . The FN (fundamental number) for both Coccinellinae and Epilachninae as recorded ranges form 14 to 20. The fundamental number, therefore coincides with that of the already worked out species. Serious discrepancies arise between visual estimates and actual measurement. Sasaki (1962) attributed some of these variation in chromosome size to the effect of colchicine. In view of these considerations attempt to identify al chromosomes of a karyotype individually were not seriously made off course,, due emphasis was given for preparing idiograms on the basis of mean chromosome length as far as possible. The diploid morphometric analysis of the karyotype including total length of the haploid complement and other essential features were taken into consideration for the present studies of coccinellids species.

Centrmeric position

The centromere on kinetochore is a specialized region of the chromosome and is associated with movement of the chromosomes along the two poles of the spindle apparatus. It is a self perpetuating organelle and has a functional role in chromosomal arrangement and chromosomal evolution (Navaschin, 1932).

From the karyotype displayed pictorially by cutting out mitotic metaphase chromosomes form photomicrograph and arranging them by length and in pairs and aliening such that the centromeres were at the same level and the short arms are oriented upwards. The chromosome of the same species have a single centromere that divides the chromosome in to two arms, the relative length of these arms are a diagnostic feature for identifying an individual chromosome. The position of centromere in the chromosome has been important determinant in the nomenclature of chromosome. The term 'acrocentric' indicates, chromosomes whose short arm is less than 1/20th of the total length i..*e* with arm ratio > 19. The arm ratio between 19 and 9 is submetacentric, the arm ratio between 9 and 1.25 being heterobrachial metacentric and ones with arm ratio between 1.25 and 1 is isobrachial metacentric (White, 1973). The present investigator has, however used the terminology of Levan ***etal.*** (1964) according to whom the arm ratio greater than 7 in called terminal (t), the acrocentric of White (1973): which between 7 and 3 is 'sub-terminal' (st), 'sub telocentric' the other term; arm ratio between 3 and 1.7 is submedian (sm), submetacentric of White and those in which it is less than that or exactly 1.0 is referred to as metacentric chromosome (m). As already mentioned that the variation of centromeric positions frequently result in variable fundamental number. This has an impact not only for morphometrical or quantitative analysis of individual chromosome but also in chromosomal polymorphism in various species within a single genus or interrelated species.

Sex chromosome

The sex chromosome with their extreme degree of diversity both in structures and behavioural patterns in various group of animals, provide a cytological criterion of an exceptional value (Sharma, 1966). They have been extensively used in cytotaxonomy of a number of animal groups where the simple and the multiple sex chromosomes exist together (Manna, 1969). The taxonomic value of sex chromosomes has been extensively furnished in various animals, providing determining mechanism. A chromatin mass called sex chromatin was present in the normal female but not in the normal male. It was evident that in most insect males have XY and female have XX. Therefore male sexes are heterozygous unlike their female counterparts.. Subsequently Morgan's (1922) investigation showed that sex linked genes are carried in the X chromosome, whereas the Y chromosome behaved as though it was empty of genes

The most characteristic and widely present type of sex mechanism in Coleoptera consists of a relatively large metacentric X and a much smaller metacentric 'y' in male (Saha, 1973). During meiosis they are associated to form a peculiar type of sex pair. In metaphase I this has been stretched into a structure resembling a " parachute" (Smith 1952) In the family Coccinellidae, the diploid chromosome with different types of sex determining mechanism viz; unequal X and y, Xy_p and neo-XY (Smith 1960). In the subfamily Epilachninae, most of the species have Xy_p type of sex mechanism (Takenouchi 1955, agarwal 1960, 1961). Yosida (1948) observed the presence of minute y chromosome in the spermatogonial cell of *E. vigintiopunctata,* though Bose (1948) found large Y, approaching the size of the X in this species. Both the sex chromosomes were metacentric, largest X was 'V' shaped and Y was metacentric . But the present study shows the presence of unequal X and y in the form of Xy where y was always minute.

Yosida (1949) reported in males of *E. orientalis* and *E. vigintioctopunctata* a translocation involving an autosome and X chromosome and the loss of minute y-chromosome which is almost near the limit of visibility might have given rise to neo-XY condition during the course of evolution of the species. The possibility, nevertheless, remains that the minute y-chromosome may have been retained not as a separate entity but by being translocated on to some other member of the complement. Mittal et al. (1989) reported that spermatogonial metaphsse X chromosome was metacentric in *E. indica,* the sex bivalent of which formed by X+Y .

The Indian species of Coccinellidae posses XY, Xy_p and neo-XY types of sex-determining mechanism (Yadav et. al. 1979). He also reported Xy_p type of sex chromosome in *E. vigintiocto punctata* (Fab) But the present study failed to revealed parachute form in X and y chromosome.

Tsurusaki *et.al.* (1993) reported X and y sex chromosomes in most males of the *E. vigintioctomacalata* complex form a typical Xy_p bivalent. An Xyy_p association was also found in some males from various population of *E pustutosa.* It is obvious that the Xyy_p association was derived from an addition of a supernumerary Y in those males. A structure similar to the Xyy_p has also been reported by Takenouchi (1968) in four out of five males of *E Niponica.* Xy_p bivalen was not identified by the authoress.

Agarwal (1961) reported, the spermatogonial cell of *Coccinella repanda* comprised on Xy complex in the form of a typical parachute. No parachute form was found in any species of the genus *Coccinella* even in any members of the Coccinellidae. X and y chromosomes were always in non homologous condition, as observed by Agarwal (1961). The Xy type of sex chromosomes also reported by Bose (1948), Agarwal (1961), Smith (1965). An acrocentric X-chromosome and the smallest spherical y chromosome were found in *C.septempunctata* Agarwal (1961). Sub-metacentric type o X chromosome in *C. septempunctata* was reported by Mittal et al. (1989).

He also reported an unequal pair of sex chromosome X and y in male *Menochilus sexmaculata.* These reports support the present findings on sex chromosomes of *C. septempunctata* and *C.sexmaculata.*

The finding of the present study are in agreement with this view that X chromosome is always larger and y is minute Smith (1949). whereas in *M. discolor* (Fab), *C. septempunctata* Linn. and *Illeis indica* Timb. it is of the Xy_p type.

The sex chromosome mechanism is simple Xy_p in majority of the coccinellids species in *Hyperspis spp.* The sex chromosome do not actually pair (X+y) Deviative neo-Xy is also frequently met with in Coccinellinae. However none of the cytologically known species of Epilachninae possesses neo-Xy.

A complex XXY: XXXX and X_1, X_2, Y_1, Y_2 have been reported in *Chilocorus stigma* and hybrids of *C. stigma* X, *C. tricycles* (Smith 1959).
Yadav et. al. (1979) studied another coccinellid beetle *Chilocorus nigritus* (Fab) where, of the sex chromosome was neo-XY of which neo-X was meta-centric and neo-Y is slightly sub-metscentric. The same type of neo-XY also he found in *B.suturalis* (Fab).

A survey on chromosome of the Coccinellidae indicates that the neo- Xy has originated by the fusion of the X with one of a pair of autosomes (Smith1959).
The authoress failed to observe neo-X, neo-Xy, XXY, XXXX and X_1, X_2, Y_1, Y_2 type of Chromosomes in the species investigated.

Dobzhansky (1941) emphasized that duplications, apart from polyploidy, is the only means by which the number of genes in the germplasm of an organism may be increased. Translocation, the exchange of the broken parts resulting in major alternations of chromosomes and have a profound interest in genetics. None of it was detected in the present findings.

The standard karyotype description based on male individual in the ladybird beetles showed little range of variations from the earlier observations. The present data agree with those described previously for *C. septempunctata,* (Yadav *et el.*, 1979 and Mittal *et al.*, 1989) *C. transversalis* (Manna and Lahiri 1972; Dua and Kacker, 1975), *I. indica (Yadav et al.,* 1974 and Dua *et.al.*, 1975) *C. sexmaculata* (Agarwal 1960, 1961 and Yadav,1979), *R taoi* (Smith1960b), *E. vigintioctopunctata* (Yosida 1948, Agarwal 1960,1961, Yadav 1979) *E.* septima (Kacker 1973), *E. pusillanima* (Saha and Manna 1971) In A. *misera,* diploid set showed a deviation from the normal karyotype of the Coccinellidae. The analysis of the chromosomes of *S. nubilis* revealed a little variation of from the record of Smith (1960b). In *M. discolor,* chromosomes numerically coincided with the findings of Mittal *et. al.*, (1989) but any report on *M. yasumatsui* during this investigation was wanting. *M. yasumatasui* exhibited a consistent diploid number of 20 which supports the typical number (2n=20) of Coccinellidae. The chromosomes in *psyllobora spp* reported by Smith (1961) Coincides with present findings.

A little deviation showed chromosome number in the present investigation ie,. *H. octomaculata* from another species *H. axyridis* reported by Smith (1961). The analysis of the Chromosome of *R. fulvescens* of the Coccinellinae revealed a diploid value of 20 unlike 18 the as reported by Smith, (1961) In the present investigation *A.pomicraspis quayumi, B. lineatus and S. tetranychi* . were supported by the modal number, but the *Jauravia pellucida* has shown a little deviation from the modal formula. The sex chromosomes of all the species studied here have been shown traditionall larger X and minute y in the form of nonsomology Xy (Smith and Virkki 1978).

This study provided evidence that although various species remarks individuality of chromosome was distinct with distinctive morphological analyses but some of the coccinellids were characterized of the coccinellids were charcterized by extraordinary range of karyotypic variation. The ratio of autosomes ranged a great diversity. In each species of the present investigation, last pair of autosome showed the minimum value ranged from 5.21% in *E. septima to* 9.8% in *A. misera,* while 1st pair autosome showed highest value ranging between 10.67% in *P.quatuordecimpunctata* to 17.10% in *A. misera.* Kacker (1993) mentioned that chromosome ratio differs in different species. These non overlapping ratios, seemingly characteristics for differentiating the species.

Amino acid contents of Coccinelldae

The present protocol was designed to determine the interrelationship among the various species of coccinellids by chromatgraphical analysis of amino acids contents in the cell of the beetles under study. But because of some technical constraints an investigation of the amino acid contents in all the 20 species was not possible. Consequently 12 of the aforesaid species namely *Illeis Indica* , *Psyllobora bisoctonotata, Cheilomenes sexmaculata, Coccinella septempunctata, C.transversalis, Harmonia octomaculata,, Micraspis discolor, M. yahsumatsui, Afidenta misera, Epilachra septima, E. pussillanima and E. vigintioctopunctata* were subjected to the chromatographical analysis for detection of amino acid contents.

Though the analytical method used in present studies is more primitive but modern facilities (e.g. amino acid Analyzer, amino acid sequencer) were not present and as such traditional laboratory method had to be adopted. Reports on the amino acid in the Coccinellidae were apparently wanting during this investigation.

Several investigations reports on the amino acids contents in the salivary glands of some insect are available (Nuoroteva, 1955, 1956; Kloft, 1960; Schaller, 1960, 1968; Anders, 1961; Miles 1967). Hori (1975) studied the amino acid contents in the salivary glands of the bugs Lygus disponsi and *Euridema ruposum.* A decrease of total free amino acids concentration towards late stages of embryonic development stages of *Culex* has been reported by Chen and Briegel (1965), indicating the probability of enhanced protein level but in *Antherea mylita*, Pant and Sharma (1976) noted a decrease in total protein contents during same period. Jolly *et al.* (1979) had reported the changes in the free amino acid composition of healthy and diseased fifth instar larvae of Tasar silk worm *Antherea mylita.* Changes in the haemolymph proteins have been reported by Fujii (1936) and Miyoshi (1959) in Bombyx mori L Guest and Wassink (1969) had reported the changes in heamolymph protein in Cabbage armyworm, *Mamestra brassicae.* The amino acid composition of erythrocruorin of *Marphysa sanguinea* has been determined by Chew ., (1967) with the help of TLC.

Pant &Unni(1978) indicated the amino acid composition of the fibres of *philosamia ricini* Boisd. are alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, lysine, leucine/isoleucine, proline phenylalanine, serine, tyrosine, threonine, tryptophan and valine. Poonia (1978) identified the amino acids content in the haemolymph of *p. ricini* Boisd. as cysteic acid, aspartic acid, serine, glycine, alanine, lysine, glutamine, methionine-sulphoxide, tyrosine, valine, leucine/isoleucine, histidine, threonine and proline. Amino acids content in the silkgland of matured larva of *Bombyx mori* L tyrosine, serine, aspartic acid, glycine, threonine, valine, cistein, alanine, glutamic acid, leucine/ isoleucine were identified by *Saha* (1982). Das (1985) revealed the presence of alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, lysine, leucine/isoleuecine, methionine, phenylatanine, proline, serine, tyrosine, thrionine, tryptophan and valine in *P ricini* Boisd. Both the wokers used paper chromatographic process. No report on the amino acids contents of Coccinellidae was available.

The present findings 10 amino acid contents of the species *Illeis indica* Timbelake were detected of which alanine, glycine, valine, proline, and phenylalanine showed higher concentration. In other species of the same tribe *Psyllobora bisoctonotata* (Mulsant) all the amino acids as in *I. indica* were detected except tyrosine.

Cheilomenes sexmaculata (Fab) showed the presence of alanine, cystine, glycine, glutamic acid, isoleucine, leucine, proline, tyrosine, and lysine which are also less in number than former one of the two species of the genus *Coccinella viz. C septempuctata* Linn showed the presence of 11 amino acids, histidine, aspartic acid, prolin, glutanic acid, luceine, alanine, glycine, phenylalanine, lysine, valine and tyrosine, while the other species *C transversalis* Fab. showed the presence of the same numbers of amino acid only the histidine was replaced by arginine. In *Harmonia Octomaculata* (Fab) 10 amino acids were identified where tryptophan was present in addition to other nine common amino acids. *Microspis discolor* (Fab) and *M yasumatsui* Sasaji differed each other with an excess amino acid threonine present in the first one out of 9 common amino acids. Under the sub family Epilachninae, of the two species *Afidenta misera* Mulsant and *Epidachna septima* Deike differeces were found in the proline and serine, prolin was absent in *E. septima,* other amino acids present in both the species were alanine, glycine, arginine, lysine, phenylalanine, leucine and valine. *E. pusillanima* Mulsant *Epilachna vigintioctopunctata* Fab showed the same kinds of amino acids which differed only in their concentration.

The present investigation showed that three amino acid the alanine, lysine and glycine were always common in all the studied species with higher concentration. In the sub family Epilachninae, the aspartic acid, glutamic acid were absent, whereas in the Coccinellinae specially under the tribe Coccinellinii, aspartic acid and glutamic acid were detected everywhere. In the species of the tribe Psylloborinii, aspargin was found in addition to other amino acids. But the aspargin was absent in other species in the present investigation.

Thus cytogenetic evidence suggest that the chromosomal rearrangement has been a direct role in speciation, a process ensued from the very early stage in the evolutionary divergence of forms of life. The phenomenon of biological complexities and adaptations are this compounded as speciation involved in accumulation of phylogenetic changes, quantitatively increasing the strength of genetic isolation until the process is complete. The whole process of organic complexities, organic diversities and adaptiveness are explained in the terms of scientific interaction as products of the process of evolution. That foregoing accounts of chromosomal rearrangement may play a significant role in speciation. Likewise, variation of the staining properties are indices of differences of gene action (Sharma, 1976). Changes in chromosomes morphology are destined to rearrange the gene sequence in the chromosomes as a result their segregation and recombination even are effected and thus the entire gamute of morphological, physiological and biochemical aspects are altered (Sharma, 1976). It needs to be recognized that identifying or defining a species in term of karyotype will not be so much rewarding as though arrangement in term of karyotype can contribute to species formation, but they are one facet to much larger and more complicated problems for which evidence from ecology, morphology and biology should be brought into consideration since evolution works at the species level and may involve any of these criteria singly or collectively at the point of threshold leading to the differentiation of a new species. (Sharma, 1966).

CHAPTER - 6

CONCLUSION

Conclusion

Structural configuration and behaviour pattern of chromosomes to twenty species of Coccinellidae belonging to sixteen genera have been investigated . Where twelve species of the possess typical karyotype 2n= 20 (9AA+Xy); four with 2n= 18 (8AA+Xy), two with 2n= 16 (7AA+Xy) and two with 2n = 14 (6AA+Xy). It referred all the species followed the modal number of Coccinellidae reported by Smith (1960) .Inter species variation has been found in different ranges of chromosomal measurements. A new species *Apomycraspis quayumi* Ali & Rahaman, studied here which is nearer to the genus Micraspis but it is only the proposed name, still now taxonomically unestablished. Though numerical structure of its chromosomes similar to that of the *Micraspis sp* but they differed in other structural ranges of chromosomes. So, it may be justified to consider it as a separate species. Amino acids detection in twelve coccinellids species. The species beloning to the same genus show close similarity as their amino acid contents. However they exhibit the differentiation from species to species. Therefore, it can be conclude that chromosomal studies along with amino acids composition have effect variation on cytogenetics of ladybird beetles (Coleoptera: Coccinellidae).

CHAPTER - 7

LITERATURE CITED

Literature cited

Agarwal, U. 1960. Chromosome number and sex mechanism in sixteen species of Indian Coleoptera. *curr. Sci.* **29:** 140-144.

Agarwal, U. 1961. Studies on the chromosomes of five species of Coccinellidae (Coleoptera). *Cytologia* **26:** 285-293.

Ahmad, R. 1968. A new species of *Pseudoscymnus* Chapin [Col., Coccinellidae] predaceoucs on scale insects in West Pakistan. *Entomophaga,* **13(4)**: 377-379.

Ahmad, R. 1970. A new species of *Pharoscymnus* Bedel [Coleoptera: Coccinellidae] predaceous on scale insects in Pakistan. *Entomophaga,* **15(3)**: 233-235.

Ahmad, R. 1973. A new tribe of the family Coccinellidae (Coleoptera). *Bull.* Entomo. Tes., **62:** 449-452.

Ahamd, R.and Ghani,M. A. 1966. A new genus and species of Chilocorini (Coleoptera : Coccinellidae) from Pakistan. *proc. R.ent .Soc., Lond* **(3)35 (1-2)**:9-10.

Alam, M. Z. 1962. *A list of insects and mites of East Pakistan.* Agr. Res. Instt. Tejgaon, Dacca. 107 PP.

Alam, M. A., Ahmed, A., Alam, S. and Islam, M. A. 1964. Coccinellidae (p. 67). In: *A review of Research Division of Entomology (1947-1964).* Agric. Infrm. Serv. Dacca, East Pakistan. 172 pp.

Alam, M. Z. 1967. Coccinellidae (pp. 22-24). In : *Areport on the survey of insect and mite fauna of East Pakistan.* East Pakistan Agricultural Research Institute, Ayubnagar, Dacca. 151 pp.

Anand, R. K., Gupta, A. K. and Ghai, S. 1988. A check-list of Indian Epilachninae (Coccinellidae: Coleoptera) with recorded host plants. *Bull. Ent.*, **29**(1):121-137.

Anders, F. 1961; Untersuchuroen uber das cecidogene prinzip der Reblaus (*Viteus vitiflii Schimer*). II. Bilogische Untersuchungen Uber das galleninduzierende Sekret der Reblaus. *Biol. Zbi.* **79.** 679-700.

Ayala. F.J. Tracey . M.L., Hedgecock, D., & Richmond, R.C. 1974. Genetic differentiation during the speciation process in *Drosophila. Evolution,* **28:** 576-592.

Ayyar, R. T. V. 1925. An undescribed coccinellid beetle of economic importance. J. Bombay nat. Hist. Soc., **30**(1): 491-492.

Baker, R. J., Koop, B. F. And Haduk, M. W. 1983. Resolving systematic relationship with G-bands: a study of five genera of the south American cricentine rodents. *Systemat. Zool.* , **32:** 403-416.

Bielawsky, R. 1972. Die Marienkafer (Coleoptera: Coccinellidae) aus Nepal. *fragm. Faun.*, **18**: 283-312.

Bielawsky, R. 1979. Ergebnisse der Bhutan-Expedition 1972 des Naturhistorischen Museums in Basel Coleoptera: Fam. Coccinellidae. *Entomologica Basiliensia,* **4 :** 83-125.

Bennet, M.D. 1965. The Karyotype of the mouse with identification of a translocation. *Proc. Nat. Acad. Sci.*, USA., **53:** 730-737.

Benzer, S. 1955. The fine structure of a genetic region in bacteriophase. *Proc. Nat. Acad. Sci.,* **41:** 344-354.

Benzer, S. 1962. Amino acid in gene sequence. *Scient. Amer.* **206:** 70.

Bose, I. 1948. The association of non homologous chromosomes in the spermatogenesis of the ladybird beetle *Epilachna vigintioctopunctata* (Fab) . *Proc. Zool. Soc. Bengal,* **1:** 131-134.

Booth, R. G. and Pope, R. D. 1989. A review of the type material of Coccinellidae (Coleoptera) described by F. W. Hope, and by E. Mulsant in the Hope Entomological Collections, Oxford.

Booth, R. G., Cos, M. L. and Madge, R. B. 1990. Coccinellidae (pp. 82-94). In: *IIE Guides toInsects of Importance to Man 3. Coleoptera.* International Institute of Entomology, The natural History Museum. 384 pp.

Booth, R. G. 1993 International Institute of Entomology, Identification Services Report, 22655/11655 Asia, 2nd February, 1993. 2 pp.

Bovery, Th. 1893. Uber die Entstehung des Gegensatzes Zellen bie Ascaris. *Sitzungber. Ges. Morph. Phys. Munchen.,* **8:** 114-125.

Brinkley, B. R. and Hittleman, W.N. 1975. Ultrastructure of mammalian chromosome aberrations. *Int. Rev. Cytol.,* **42:** 49-101.

Britton , G. Lockley W.L.S. Harriman G.A Goodwin T.W. 1977. Pigmentation of the ladybird beetle Coccinella septempunctata by carotenoids not of plant origin. **266:** 49-50.

Britton-Davidian, J. 1990. Variabilite genetique chez les populations de soures (genre *Mus* L.) a nombre chromosomique reduit. *Competes Rendus de L, Academie des science,* paris, **290:** 195-198.

Brown, S. D. M. 1984. Concerted evlution and molecular drive: Evolutionary sequence change. In : *Genetics : New Frontiers* (V.L. Chopra *et al.,* eds.) pp. 221-134. oxf. & IBH. New Delhi.

Bugenberg, de Jong, C. 1957. Polyploidy in animals. *Bibliog. Genet.*, **17:** 111-228.

Capanna, E., Civitelli, M. V. and cristald, M. 1977. Chromosomal rearrangement, reproductive isolation and speciation in mammals. The case of *Mus musculus. Bolletino di Zoologia,* **44:** 213-246.

Casey, T. L. 1899. A revision of the American Coccinellidae. *J. New york entomol. Soc.*, **7:**71-169.

Chapin, E. A. 1940. New genera and species of lady-beetles related to *Serangium* Blackburn (Coleoptera: Coccinellidae). *J. Wash. Acad. Sci.,* **30** (6): : 263-272.

Chapin, E. A. 1962. *Pseudomcymnus,* a new genus of Asiatic Scymnini (Coleoptera: Coccinellidae). *Psyche,* **69** (1) : 50-51.

Chapin, E. A. 1965a. The genera of the Chilocorini (Coleoptera, Coccinellidae). *Bull. Mus. Cmp. Zool., Harvard Univ.,* **133** (4) : 227-271.

Chapin, E. A. 1965b. Insects of Micronesia Coleoptera: Coccinellidae. *Insects of Micronesia,* **16**(5): 189-254.

Chapin, J. B. 1974. The Coccinellidae of Louisiana (Insecta: Coleoptera). *Louisiana state Univ. Agr. Exp. Sta. Bull.*, **682**: 1-87.

Chen, P.S. and Briegel, H. 1965; Studies on protein Metabolism of *Culex* L.V. Changes in free amino acids and peptides during embryonic development, *Comp. Biochem. Physiol.*, **14:** 463.

Chew, M.Y. 1967. Amino Acid Composition of *Marphysa sanguina* Erythrocarocruorin *I.J. of Biocham* **5:** 1.

Chowdhury, K.A. 1970. M. Sc. Thesis, Department of Biochemistry, University of Dhaka, Dhaka.

Clark, J.M. 1963. *Experimental Biochemistry.* W.H. freeman and company, U.S. A., pp . 228.

Clausen, C. P. 1965. Biological control of insect pests in continental United States. *USDA Tech. Bull.* 1134, 151 pp.

Crotch, G. R. 1874. *A Revision of the Coleopterous Family Coccinellidae'.* (Janson: London.) 311pp.

Crozier. R.H. 1968. An acitic acid dissociation Air–drying technique for insect chromosome. with acito-lactic orcein staining. *Stain tech.* **43:** 123-134.

Darlington, C.D. 1937. *Recent advance in Cytology.* 2nd. ed. Blackiston son & co., Phila., 671p.

Das, R.R. 1985. Amino acid detection from *P. ricini.* M. Sc. thesis . Dept. of Zoology. R.U. (Unpublished).

Dasgupta J. And Chakrabarti A 1972a. Cytological studies on the six species of Chry somelidae (Insecta: Cloeoptera). *Curr. Sci* **41:** 490.

Dasgupta J. And Chakrabarti A 1972b. Chromosome some complement in seven species of Indian Garabidae (coleoptera). *Indian Biologist* **4:** 60.

Dasgupta J . and Chakrabarti A 1973 Chromosome some numbers in twelve species of Indian Carabidae and tenebrionidae (Coleoptera) Curr. Sci. **42:** 102.

Dasgupta, J. 1977. Comparative cytology of seven families of Indian Coleoptera. *the nucleus* vol **20 (3) :** 294 – 301.

Dieke, G. H. 1947. Ladybeetles of the genus *Epilachna* (Sons. Lat) in Asia,Europe and Australia. *Smithson.Misc.* **Coll.106(5) :** 1 –183.

Dobzhansky, T. 1925. Zur Kenntnis der Gattung *Coccinella* auct. *Zool. Auz.,* **62:** 241-249.

Dobzhansky, Y. 1941. Genetical and cytological proof of translocations involving the third and fourth chromosomes *of Drosophila melanogaster. Biol. Zentralbl.*, **49:** 408-419.

Drilhon, A., Busnel, R. G. and Vago, C. 1951; less acids amines libres et les substances fluores centes dusang et des tubes de Malpighi, de la chenille de *B. mori* L;, ate inte dela maladie a poly dresset de la flacherie, *Compt. rend. acad. Sci.* **232:** 360-361.

Drilhon, A., Busnel, R. G. and Vago, C. 1952. Essai de diagnestic precocdela grasserie et dela flacherie per l' analysis chromatographique du sang de *B. mori L. Compt.red. spc. Boil.* **146:**11-12.

Dua., P.S and Kacker R.K. 1975. Chromosome number in ten species of Coleoptera. *Newsl. Zool. Surv. India* **1:** 32.

Dua, P.S. and Kacker, R.K. 1976. Chromosome number in Indian Coleoptera. *Rec. Zool. Surv. India,* **2:** 88-89.

Fabricius, J.C.1798.*Supplementum Entomologiae Systematica,* Hafniae, 572 pp.

Florencio, I. S. , Saidul, I. 1990. Cytological analysis of mitotic and salivary gland chromosomes of *Dacus (bacterocera) dorsalis* Hendel. Philipp. *Nucl. J.* **vol. 7.**

Flemming, I. S. 1879. Cross reference of Verma, P.S. and Agarwal, V.K. 1981. Cytology. *Chand and Co. Ltd.* Delhi, 85 pp.

Ford, E.H.R. and Wollam, D.H.M. 1963. A study on the mitotic chromosomes of mice of the strong line, *Exp. Cell Res.,* **32:** 320-326.

Ford , E. B. ,!964. Ecological Genetics . Methum, London, *J. Willey*, Newyork. 335pp

Fowler, W W. 1912. *The Fuana of British India, including Ceylon and Burma, Coleoptera. General Introduction and Cicindelidae and pussidae.* Taylor and Francis, London. 529 pp.

Fujii, o. 1936. Biochemical studies on silkworm flacherie, *Rept. Sericult. Exp. Biol.* Perfect No. **6:** 1-77.

Garber, E.D. 1978. Cytogenetics in cell Biology : A Comprehensive Treaties, (L. Goldstein and D.M. Prescot, eds.), **Vol. 1.** academy press, New York.

Ghani, M. A. 1962. A note on the identity of some species of the genus *Ballia* (Coleoptera: Coccinellidae). *Proc. R. Ent. Soc. Lond.* , **31** (B) : 92-93.

Gill, T. K., Mittal, O.P. and Gupta, S. 1988. Metacentric sex Chromosome in a rodent species, *Rattus (Millardia) Meltada (Ryley). Cytobios.*, **55:** 185-190.

Gordon, R. D. and Chapin, E. A. 1983. A revision of the New World species *Stethorus Weise (Coleoptera: Coccinellidae). Trans. American Entomol. Soc.*, **109** : 229-276.

Goldjman, I.I., Smerttenko, I.I. and vilkina, 1966. The normal karyotype in mice of a highly leukemic AKR strain. *Bull. Eksp. Biol. Med.*, **61:** 75-78.

Gorham, H. S. 1894a. On the Coccinellidae from India in the collection of Mr. H. E. Andrewes of the Indian Forest service. *Ann. Soc. entomol. Belgique.*, **38**(5) : 200-208.

Gorham, H. S. 1894b. Insects du Bengale (32 ^e^ Memoire). Observations on the species of the family Coccinellidae collected near Konbir and Mandar, India, Bengal by p. Cardon. *Ann. Soc. entomol. Belgique.*, **38** : 209-211.

Grosse-Ruschkamp, A. 1994. *Integrated pest Mangaement Guidelines.* Deutsche Gesellschaft fur Technische Zusammenarbe it (GTZ) CmbH, Germany. 119 pp.

Guest, S. V. and Wassink, N. G. 1969. Changes of haemolymph protein in cabbage armyworm, *Mamestra brassicae. J. Insect physiol.* **16:** 187-195.

Guignard, L. 1891 Nouvelles e'tudes Sur La fecondation. *Ann. Sci. Nat. Bot* **69:** *491-514.*

Hans, G. Schelegel 1986. General Microbiology. *Cambridge univefsity Press.* **7:** 7-16.

Heitz, E.1928.Das heterochromatin der Moose. *I. Jahrb. Wiss. Bot.,* **69**:762-818.

Heitz, E. 1932. Die Herkunft der chromoscentren (eng.ed.). *Planta,* **18:** 571-636.

Hemptinne, J. L., Lognay, G., Doumbia, M.& Dixon, A.F.G. 2001) Chemical nature and persistence of the ovipostion deterring pheromone on the tracks of the larvae of the two spot lady bird , Adalia bipunctata. (Coleoptera: Coccinellidae). *Chemoecology* **11:** 43-47.

Hemptinne, J.L. Lognay, G., Gauthier , C. & Dixon, A.F.G. *(2000)* Body size distribution in predatory ladybird beetles reflects that of thir prey. *Ecology* **82:**1947-1845.

Hemptinne, J.L. & Dixon . A F.G. (2000) Defence, oviposition and sex: semiochemical parsimony in two species of ladybird beetles (Coleoptera, Coccinellidae) A short review. *European Journal* of *Entomology* **97:** 443-447.

Hemptinne, J.L., Dixon . A F.G. & Gauthier, C., 2000. Nutritive cost of intraguild predation on eggs of Coccinella septempunctata and Adalia bipunctata Coleoptera. : Coccinellidae.) *European Journal of Entomology* **97:** 443-447.

Hodek, I. 1973. *Biology of Coccinellidae.* Academia, Czechoslovak Academy of Science. 260 pp.

Hodek, I & Honek, A 1999. Ecology of Coccinellidae *series entomologis* **54** : 26-34.

Hori, K. 1975 Amino acids in the salivary glands of the Bugs, *Lygus disponsi* and *Eurydema rugosum. Insect Biochem.*, **5:** 165-169.

Hoy, W.E. 1918. A study of somatic chromosomes. 11. *Biol., Bull*, **35:** 166-174.

Hsu, T.C. 1973. Chromosome identification. *Nobel Symp.*, **23:** 32.

Hsu, T.C. and Patton, S. 1969. *Chromosoma.* **58:** 269.

Hsu, T.C. and Pomerate, C.M. 1953. Mammalian chromosome in vitro. II : A method for spreading the chromosome cells in tissue culture. *J. Hered.*, **44:** 23-29.

Hubby, J.L. & Throckmortion, L. H. 1967. Protein differences in *Drosophila,* IV. A study of sibling psecies. *Amer, Naturalist,* **102:** 193-205.

Iablokoff- Khnzorian, S. M. 1979. Genera der palaarktischen Coccinellini (Coleoptera: Coccinellidae). *Entomologische Blatter fur Biologie und systematic der kafer,* **75 :** 37-75.

Iablokoff-Khnzorian, S. M. 1982. *Les Coccinelles Coleopteres- Coccinellidae Triby Coccinellini des regions Palearctique et Orientale' Paris.* 568 pp.

Imai, H.T. 1966. The chromosome observation techniques of ants and the chromosomes of Formicinac and Myrmicinae. *Acta Hymenopterologica,* **2:** 119-131.

Imms, A. D. 1977. *Recent Advances in Entomology.* Mathuen and Co. London. 886 pp.

Ishimori, N. and Muto, M., 1951. Free amino acids in the blood of the normal grasseric silkworm (B. mori, L) by paper chromatography. *J. Sericult Sci.* **20:** 128-131.

Jolly, M.S., Sen, S.K ., Son Walker, T.N. 1979. Non-mulberry silks, FAO agril, Seri, Bull. FAO, Rome. P. 178.

Kabir, A. K. M. F. 1975. Natural enemies of jute pests (p. 56-57). In: *Jute pests of Bangladesh.* Bangladesh Jute Research Institute, Sher-e-Bangla Nagar, Dacca. 59 pp.

Kacker, R.K. 1976. Studies on the chromosomes of Indian Coleoptera.VI. Chromosome number and sex determining mechanism in 15 species of Coleoptera. *Newsl. Zool. Surv. India*, **2:** 48-49.

Kacker, R.K. 1973. Studies on the chromosomes of Indian Coleoptera.V. on the cytology of *Hoplobrachium asperipenne* fairm. (Tenebrionidae: Amarygminae), *Epilachna septima* Diek. (Coccinellidae: Epilachninae), *Caryedon gonagra* (Bruchidae: Bruchinae). *Cytologia* **38:** 535-538.

Kacker, R.K. 1993. Chromosome and phylogeny of coleopter, *Zoological survey of India Calcutta* **147:** 1-35.

Kamiya, H. 1959. On the identity of *Stethorus punctillum* of Japanese authors (Coleoptera: Coccinellidae). *Kontyo,* Japan **27:** 139-143.

Kamiya, H. 1960. A new tribe of Coccinellidae (Coleoptera). *Kontyo,* Japan **28** (1) : 22-26.

Kamiya, H. 1961a. A revision of the tribe Scymnini from Japan and the Loochoos (Coleoptera: Coccinellidae). I. Genera *Clitostethus, Stethorus* and *Scymnus* (except subgenus pullus) *J. Fac. Agr. Kyushu Univ.* **11** (3) : 275-301.

Kamiya, H. 1961b. A revision of the tribe Scymnini from Japan and the Loochoos (Coleoptera: Coccinellidae). Part II. Genus *Scymnus* (Subgenus *pullus). J. Fac. Agr. Kyushu Univ.,*

Kapur, A. P. 1940. A note on the lady-bird beetles (Coccinellidae) predating upon the cane white –fly, *Aleurolobus barodensis* Mask. *Curr. Sci.,* **9** (3) : 134.

Kapur, A. P. 1942. Bionomics of some Coccinellidae, predaceous on aphids and Coccids in North India. *Indian J. Ent.*, 4 (1) : 49 - 66.

Kapur, A. P. 1943. On the biology and the structure of the coccinellid *Thea bisoctonotata* Nuls. in Noth India. *Indian J. Ent.*, 5 (1-2): 165-171.

Kapur, A. P. 1946. A revision of the genus *Jauravia* Mots. Coleoptera Coccinellidae). *Ann. Mag. nat. Hist.*, **13** (2): 73 - 93.

Kapur, A. P. 1948a. On the old World species of the genus *Stethorus* Weise (Coleoptera, Coccinellidae). *Bull. Entomol. Res.*, 39(2): 297-320.

Kapur, A. P. 1948b. A revision of the tribe Aspidimerini Weise (Coleoptera-Coccinellidae). *Trans. Royl. Entomol. Soc. Lond.*, **99.** 77-128.

Kapur, A. P. 1948c. The genus *Tetrabrachys* (ILithophilus) with notes on its biology and a key to the species (Coleoptera: Coccinellidae). *Trans. Royl. Entomol. Soc. London.*, **99**(9) : 319-339.

Kapur, A. P. 1949. On the Indian species of *Rodolia Mulsant I* (Coleoptera-Coccinellidae). *Bull. Entomol. Res., 39* (4): 531-538.

Kapur, A. P. 1950. A new species of *Stethorus Weise* from India (Coleoptera: Coccinellidae). *Proc. R. Ent. Soc. Lond.*, (B) **19:** (1950) 148-149.

Kapur, A. P. 1951a. Further notes on the Indian Species of *Rodoia* Mulsant (Coleoptera: Coccinellidae). *Rec. Indian Mus.*, 48:1-7.

Kapur, A. P. 1951b. Expedition to South-West Arabia, 1937-38. No. 18-Coleoptera: Coccinellidae. British Museum (Nat. Hist). London, pp. 275-297.

Kapur, A. P. 1956a. A new species of Coccinellidae (Coleoptera) predaceous on the citrus white –fly in India. *Rec. Indian Mus.*, **52** (2-4): 189-193.

Kapur, A. P. 1956b. Systematic and biological notes on the ladybird beetles predacious on the San Jose scale in Kshmir with description of a new species (Coleoptera: Coccinellidae). *Rec. Indian Mus.*, **52**(1954): 257-274.

Kapur, A. P. 1958. Coccinellidae of Nepal. *Rec. Indian Mus. Calcutta*, **53** (1955): 309-338.

Kapur, A. P. 1961a. A new species of *Stethorus* Weise (Coleoptera –Coccinellidae). feeding on arecanut palm mites in kerala, Southern India. *Entomophaga*, **6** : 35-38.

Kapur, A. P. 1961b. A new species of the genus *Jauravia* Mots. from India (Insecta : Coleoptera: Coccinellidae). *Rec. Indian Mus.*

Kapur. A. P. 1963a. The Coccinellidae of the Third Mount Everest Expedition, 1924 (Coleoptera). *Bull. British Mus. Nat. Hist. (Ent.)*, **14** (1):1-28.

Kapur. A. P. 1963b. Confirmation of the occurrence of the lady-beetle, *Coelophora puillata*(Swartz) (Coccinellidae, Coleoptera), in India and its biological importance. *Sci & Cult.*, **29**(5): 264.

Kapur. A. P. 1966. The Coccinellidae (Coleoptera) of the Andamans. *proc. Nat. Inst. Sci. India*, **32B** (3&4): 148-189.

Kapur. A. P. 1969. On some Coccinellidae of the tribe Telsimiini with descriptions of new species from India. *Bull. Syst Zool., Calcutta*, **1** (2) : 45-56.

Kapur, A. P. 1973. On a collection of lady-bird beetles (Coccinellidae, Coleoptera) from Bhutan. *orient. Ins.*, **7**(3) : 457-460.

Katakura , H. 1976. Phenology of two sympatric phytophagous ladybirds of *Henosepilachna vigintioctomaculata* complex in and near Sapporo, northern Japan (Coleoptera : Coccinellidae). *J. Fac. sci.* Hokkaido Univ,Ser VI, Zool. **20:** 313 – 328.

Katakura, H. 1981. Classification and evolution of the phytophagous ladybirds belonging *Henosepilachna vigintioctomaculata* complex (Coleoptera : Coccinellidae) *J. Fac. sci.* Hokkaido Univ,Ser VI, Zool 22: 310 – 378.

Katakura, H. 1982. Long mating season and its bearingon the reproductive isolation in a pair of sympatric phytophagous ladybirds (Coleoptera : Coccinellidae). *Kontyu* (Tokyo) **50:** 599 – 603.

Katakura, H., 1986. Evidence for the incapacitation of heterospecific sperm in the female genital tract in a pair of closely related ladybirds (Insecta, Coleoptera, Coccinellidae). *Zool. Sci.* **3:** 115 – 121.

Katakura, H. 1988. Insects in Japan. *Epilachna vigintioctomaculata* complex *Bun-ichi Sogo* Press, Tokyo, **10:** 159.

Katakura, H. , Shioi M. , Kira Y. 1989. Reproductive isolation of host specificity in a pair of phytophagous ladybird beetles. *Evolution* **43:** 1045 – 1053.

Katakura, H. 2001. Comparison of genetic variation in growth performance of *Epilachna vigintionctomaculata. Heridity* **87:** 1-7.

Kitzmiller, E. L. 1976. Trends of populations of aphid predators in South Dakota. *Ann. ent. Soc.* Am. **60:** 516-618.

Kloft, W. 1960. Wechselwirkungen Zwischen pflanzensaregenden insekten und den vonihnen besogenen pflanzengenweben. *Teil I.Z. angew. Ent.* **46 :** 337-381.

Komai, T. 1956. Genetics of ladybeetles. *Adv. Genet.* **8:** 155-185.

Korschefsky, R. 1930. *Coleopterum Catalogus pars* 118. Coccinellidae I. 224 pp. Berlin.

Korschefsky, R. 1933. Bemerkungen uber Coccinelliden von Formosa. *Trans. Nat. Hist. Soc. Formosa,* **23** (128 & 129) : 299-304.

Kuboki, M. 1978. Studies on the phylogenetic relationship in *Henosepilachna vigintioctomaculata* complex based on variation of isozymes (Coleoptera: Coccinellidae). *Appl. Entom. Zool.*, **13:** 250-256.

Lahiri M. And Manna GK. 1969. Chromosome complement and meiosis in forty –six species of Coleoptera. *C.L. S.* **13:** 9.

Latreille, P. A. 1810. *'Consideration Generales sur l' Ordrenaturel des Animaux co,posant les classes des Crustaces, des Arachnides et des Insects.* (F. Schoell: Paris.)

Lehninger, A.L. 1972. *Biochemistry,* worth publishers, Inc; New York, N.Y. 10011; P.67.

Levan, A., Hsu, T.C. and Stich, 1962. The idiogram of the Mouse. *Hereditas.*, **48:** 677-687.

Levan,A., Fredga, K. and Sandberg, A.A 1964 . Nomenclature for centromeric position on chromosomes. *Hereditas,* **52:** 201-220.

Lewis, K.R. and John, B. 1963. *Chromosome Marker.* Churchill, London.

Lewitsky, G.A. 1924. *The material Basis of heredity.* Kiev, State Publication office of the Ukraine. pp. 166.

Lewitsky, G.A. 1931. The Karyotype in systematics. *Bull. Appl. Bot. Genet. Plant Breeding,* **27**(1) : 187-240.

Li, J. 1940. The chromosomes of Lady –bird beelte, *Harmonia oxyridis* pallas. *peking Nat. Hist. Bull.* **15:** 157-160.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, Secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymies, locis, Ed. 10, **Vol. I**. 823 pp. Holmiae.

Mader, L. 1926-1937. Evidenz der palaarktischen Coccinelliden und ihrer Aberationen, in wort und Build. I. Epilachnini, Coccinellini, Halyziini, Synonichini XII+412,64T.15 fig. (1926-1934, wien, verein Naturbeobachter und Sammb. , 1935. *Ent. Anzeig.* , **15:** 329-383. *Ent. Nachr.* **Bl. :** 384-412).

Magro, A., Hemptinne, J,-L., Codreanu, P, Grosjean, s. & Dixon, A.F.G. 2002. Does the satiation hypotesis account for the differences in efficacy of coccidophagous and aphidophagous ladybird beetles in biological control? A test with Adalia bipunctata and Cryptolacmus. *Montrozieri, Biocontrol* **47:** 537-543.

Majerus, M.E.N. 1985. Some notes on ladybirds from an acid heath . *Bulletin* of the *Amateur Entomologists,* '*Society* **45**: 31-37.

Majerus, M. E N., Ireland, H. and Kearns, P. W. E. 1987. Description of a new form of *Adalia bipunctata* with notes on its inheritance . *Entomologist's record and Journal of variation.* **99:** 255 –257.

Majerus, M. and Kearns, P. 1989. *Ladybirds.* Richmond publishing Co. Ltd. 103 pp.

Makino, S. 1951. *An atlas of the chromosome number in animals,* 2nd edn. Iowa State College Press. 290pp.

Manna, G.K. 1969. Presidential Address, Sec. of Zoology. In: *Proc. 56the Indian Sci. Cong., Part* **2:** 185.

Manna, G.K. and Lahiri, M. 1972. Chromosome complement and meiosis in forty six species of Coleoptera. C.I.S. **13:** 9-11.

Matthey, R. 1958. Les Chromosomes des maumiferes eutheriens: Liste critique et essai Sur L evolution chromosomiaue. *Arch. J. Klaus Stift,* **33:** 253-297.

Miles, P.E. 1967. Studies on the Salivary physiology of Plantbugs: Transport from haemolymph to saliva. *Insect physiol.* **18:** 1787-1801.

Mittal. O.P. Gill. T.K. and Daid. P. 1989. Karyological investigations on five Indian Coccinellids (Coleoptera). Perspectives in Cytology and Genetics. **6:** 383-88.

Miyatake, M 1959. A contribution to the coccinellid-fauna of the Ryukyu Islands (Coleoptera). *Mem. Ehime Univ.*, **4** (6) : 125-161.

Miyatake, M. 1961a. The East-Asian Coccinellid-beetles preserved in the California Academy of Science, Tribe platynaspini. *Mem. Ehime univ.*, **6**(6): 67-86.

Miyatake, M. 1961b. A new genus of the Coccinellidae from Japan and the Ryakyu Islands (Coleoptera). *Mem. Ehime Univ.*, ., 6(6): 133-177

Miyatake, M. 1970, The East –Asian Coccinellid beetles preserved in the California Academy of Sciences tribe Chilocorini. *Mem. Coll. Agr. Ehime Univ.*, **14** (3): 303-340.

Miyatake, M. 1980, Notes on the Coccinellidae from Kinabalu, Nothe Borneo (Coleoptera), I. *Trans. Shikoku Ent. Soc.*, **15** (1-2): 41-48.

Miyoshi, J. 1959. Physiological studies on the flacherie of the silkworm *Bombyx mori* L. The content of fre amino acids and the refractive index of body fluid in flacherie. Diseases. Starved silkworm, *J. Seicult. Sci Japan* **28:** 94-97.

Morgan, L. V. 1922. Non-criss-cross inheritance in *Drosophila melanogaster. Biol. Bull.*, **42:** 267-274.

Motschulsky, V. de. 1866. Essai d' un catalogue des insects de l' ile Ceylan. *Bull. Soc. Hist. Nat. Moscow*, **39 :** 422-426.

Muggleton, J. 1978 . Selection against the melanic morphs *of Adalia bipunctata* (two spot ladybird) : a review and some new data . *Heredity,* 40: 269-280.

Mulsant, E. 1846. *Historie Naturelle des Cleopteres de France, Sulcicolles-Securipalpes, Paris.*

Mulsant, E. 1850. Species des Coleopteres trimeres secutipalpes. *Annales des Sciences physiques et Naturelles, d' Agriculture et*

Mulsant, E. 1853. Supplement a la monographie des coleopteres trimeres securipalpes. *Annales de Societe Linnaeenne de Lbon(N.S),* **1** : 129-333. [Opusc. Ent. **3**:1-205]

Navaschin, S. 1912. Sur le dimorphisme nucloeaire des celluse lomtique de Galtonia candicans. *Bull. Acad. Imp. Sci. st. pittersburg ser,* **5,6** : 373-385.

Navaschin, Jr. M. 1925. Morphologiche Jurnstudien der crepis-Arten in Bezug auf die Artbidung, Z. Zellf. *Mikrosk. Anat.,* **2:** 98-111.

Navaschin, Jr. M. 1932. The dislocation hypothesis of evolution of chromosome numbers. *Z-indukt. Abst. U. verb l.,* **63:** 224-231.

Nuorteva, P. 1955. On the nature of the plant inuiring salivary toxins on insects, *Ann. ent. Fenn.* **21:** 33-38.

Nuorteva, P. 1956. Studies on the effect of the Saivary Secretions of some Heteroptera and Homoptera on the plant growht. *Ann. ent. fenn.* **22** : 108-117.

Oldroyd, H. 1970. *Collecting, preserving and studying insects.* New york Macmillan Co., 336 pp.

Pant, R. And Sharma, K. K. 1976. Variation in glycogen, total carbobydrates, free sugars, proteins, total amino acids and some enzymes during embryonic devlopment of *Antheraca mylitta, curr. sci.* **45:** 125.

Pant, R. and Unni, B. 1978. Amino composition of the fibres of some silkworm species, *Philosamia ricini, Antherea mylitta* and *Attacus attacus*, **47(18):** 681-682.

Patton, J. L. and Sherwood, S. W. 1982. Genome evolution in the pocket gophers (genus Thomomys). I. Heterochromatin variation and speciation potential. *Chromosoma*, **85:** 149-162.

Poonia, F.S. 1978. Haemolymph proteins in fifth instar larvae of eri-silkworm. *Philosamia ricini* Boisd. after infection with flacherie disease. *Ind. J. ser.* **18**(1): 43-47.

Pope, R. 1978. Ladybird, Ladybird. *The International Wild life Magazine* 50p September. pp. 416-421.

Pope, R. D. 1988. A revision of the Australian Coccinellidae (Coleoptera) Part 1. Subfamily Coccinellinae. *Invertebr. Taxon.*,**2:** 633-735.

Prist, J. E. 1969. *Cytogenetics*. Lea and Febiger, Philadelphia.

Rabindra, R. J. 2002. Biosystematic studies on Indian predatory Coccinellidae Biocontrol. *Newsletter* . **vol.XI** : 2.

Rahman, A. S. M. S. 1991. Life history and feeding behaviour of *Micraspis discolor* Fab. (Coleoptera: Coccinellidae), a predator of aphids. IJ. Asiatic Soc. Bangladesh Sci., **17** (1) : 45-48.

Rahman, M. J. and Ali, M. S. 1992. A new species of the genus Nephus from Bangladesh (Coleoptera: Coccinellidae). *J. Asiatic Soc. Bangladesh Sci., (Communicated).*

Rahman, M. J., 1995. Review of the ladybird beetles (Coleoptera:Coccinellidae) in Bangladesh. Ph.D. thesis, Dept. of Zool. R. U. Bangladesh (Unpublished).

Rahman, M. J., Ali, M. S. and Mannan, M. A. 1995. Comments on "Coccinellid beetles of Chittagong –A Check List (Coleoptera: Coccinellidae)". *Bangladesh J. Zool.*, **23**(1) : 123-124.

Roache, L. C. 1960. Ladybird, Ladybird, Ladybird: What's in a Name? Coleopt. Bull., **14:** 21-25.

Robertson, W. R. B. 1916. Chromosome studies I. Taxonomic relationship shown in the chromosomes of Tettigidae and Acrididae. *J. Morph.*, **27:** 179-331.

Rabindra, R. J. 2002. Biosystamatic studies on I ndian predatory Coccinellidae. Biocontrol Newsletter. **vol.XI** No.2

Robinson, R. 1972. *Gene Mapping in Laboratory Animals, II.* pp. 281-323. Plenum Press, London, New York.

Saha. A.k. and Manna G.K. 1971. Cytological investigations of Indian coleoptera insects (Beetles). *Proc. 58th Ind. Sci. Congr.* Pt. **IV.** 20.

Saha. A.K. 1973. Chromosomal studies of the Indian Coleoptera. *Cytologia* 38: 363.

Saha, A.K. 1982. M.sc thesis on detection of amino acid in *B. mori.* (Lepidoptera).Rajshahi University, Bangladesh. (Unpublished).

Sasaji, H. 1967. A revision of the Formosan Coccinellidae (1) the subfamily Sticholotinae, with a establishment of a new tribe (Coleoptera). *Etizenia,* **25:** 1-28.

Sasaji, H. 1968a. Phylogeny of the family Coccinellidae (Coleoptera). *Etizenia,* **35:** 1-37.

Sasaji, H. 1968b. Coccinellidae collected in the paddy fields of the orient, with descriptions of new species (Coleoptera). *Mushi,* **42** (9) : 119-132.

Sasaji, H. 1971. *Fauna Japonica. Coccinellidae (Insecta: Coleoptera).* Tokyo, Academic press of Japan. 340 pp.

Sasaji, H. 1974. Comparison of esterase Isozymes in *Henosepilachna Vigintioctomaculata-complex* (Coleoptera). *Zool. Mag., Tokyo*, **83:** 442.

Sasaji, H. 1981. Biosystematics on *Harmonia axyridis-complex* (Coleoptera: Coccinellidae). *Mem. Fac. educ. Fukui Univ. Ser. II.*, **(30)** 59-79.

Sasaji, H. and Hisano., K. 1977. Genetic study on esterase-allozymes at two loci in *Harmonia axgridis* (Insecta: Coleoptera). *Zool. Mag., ToKyo*, **86:** 540.

Sasaji, H. and Ohnishi, E. 1973a. Disc electrophoretic study of esterase in ladybirds. Mem. fac. Educ. Jukui Univ., Ser. II., **(23):** 23-32.

Sasaji, H. and Ohnishi, E. 1973b. Intraspecific enzyme polymorphisms in *Harmonia axyridis* Pallas and its sibling species with their species specificity. *Zool. Mag,. Tokyo*, **82:** 340.

Sasaji, H. 1994. The Formosan Coccinellidae (Coleoptera) collected by the late Dr. K. Baba (Third Edition). *Spec. Bull. Essa ent. Soc.*, (2): 235-240.

Sasaki, M. 1962. Observation on the modification in size and shape of chromosome due to technical procedure. *Chromosoma*, **16:** 637-651.

Schaller, G. 1960. Untersuchung uber den anino sauregehalt des Speicheldrusense kretes der reblaus (*viteus phylloxera vitifolii* schimer), Homoptera. *Entomologia exp. appl.* **3:** 128-136.

Schaller, G. 1968. Biochemische analyse des aphidenspcichels und seine Bedeutung fur die gallenbildug. *Zool. b. (physiol.)* **74:** 54-87.

Sharma G.P. Prashad R and Paul M.K., 1959. Chromosomal dimorphism in *Coccinella septumpunctata* (Coccinellidae : Coleopptera). *Res. Bull. Panjab Univ.* **10:** 218.

Sharma, G. P. 1960. Chromosome in relation to Taxonomy and phylogenetic relationship of the animals. *Presidential Address, 53rd Indian Nat. Cong., Chandigarh.*

Sharma, A. K. and Sharma, A. 1965. *Chromosome technique : Theory and practice.* Butterworth, London, 474pp.

Sharma,G .P. 1966. *Presidential Address, 53rd Indian Sci. Cong., Chandigarh.India.*

Sharma, A. 1976. *The chromosomes.* Oxford and IBH Publishers Co., New Delhi.

Sharma, A. K. 1984. Chromosome structure as revealed through recent technique. In: *Perspectives in cytology and Genetics (eds.)* (G.K. Manna and U. Shinha, eds.), **4:** 9-13.

Shiwago, P.I. Z. 1931. Cited from Akbar, K. M. 1995. Cytological studies of the rats and mice in Bangladesh, Ph. D. Thesis , IBSc. Rajshahi university, Bangladesh. Unpublished.

Sicard, A. 1907. Coleopteres Coccinellides du Japan, recuillis par M. Harm and et Gallois. *Bull. Mus. hist. nat. paris* 1907:211.

Slizynsky. B. M. 1955. Chiasmata in the male mouse. *J. Genet.*, **53:** 597-605.

Smith, S.G. 1949. Evolutionary changes in the sex chromosomes of Coleoptera. I. Wood borers of the genus Agrilus. Evolution **3:** 344-357.

Smith, S.G. 1952 The cytology of some Tnebrionid beetle(Coleoptera). *J. Morpl.* **91:** 325-363.

Smith, S.G. 1953a. Chromosome numbers of Coleoptera. (Contains a comlete list of references up to 1953). *Heridity* 7:31-48

Smith, S.G. 1953b . The cytology of *Zopherus haldemani* Salle (Coleoptera: Tenebrionidae) and the panorpoid complex of orders. *Heridity* (MS) in smith's *Heridity* 7(1) : 31-40.

Smith, S.G. 1957a. Co,parative cytology of Chilocorini (Coleoptera). *proc. Gen. soc. Canada*, **2:** 42.

Smith, S.G. 1957b. Chromosomal evolution in Chilocorus stigma: An exception to " Robertsons" "Law" *Records Gen. Soc. Amer.* **26:** 396.

Smith, S.G. 1957c. Adaptive chromosomal polymorphism in Chilocorus stigma. proc. Gen. Soc. Canada, **2:** 40 - 41.

Smith, S.G. 1959. The cytogenetic basis of speciation in Coleoptera. *Proc. X intern. Congr. Genet.* (1956). **1:** 444 - 450.

Smith, S.G. 1960a. Chromosome numbers of Coleoptera. II. Can J. *Genet. Cytol.*, **2:** 67-81.

Smith, S.G. 1960b. Cytogenetics of Insects. *Ann.Rev. Entom*, **5:** 69-84.

Smith, S.G. 1962. Cytogenetic pathways in beetle speciation. *can. Entom.*, **94:** (9): 941-955.

Smith, S.G. 1965. Cytological species-separation in Asiatic *Exochomus* (Coleoptera: Coccinellidae): *Can. J. Genet. Cytol*, **7:** 363-373.

Smith, S.G. and Virkki, N. 1978: Animal cytogenetics, **Vol 3:** *Insecta 5*, Edited by B. John Gebriider Borntraeger Berlin. Stuttgart pp. 1-366.

Stebbins, G.L. (ed). 1950. *Variation and Evolution in Plants.* Oxford Univ. Press, pp. 80-81.

Stebbins, G. L. 1964. *Variation and Evolution in Plants. Oxford Univ. Press,* **I:** pp. 442.

Stebbins, G.L. 1966. Chromosomal variation and evolution. *Science,* **152:** 1463-1469.

Stevens, N.M. 1906. Studies in spermatogenesis,II *Carneg. Inst. Wash.* Pub. No. 36, 33.

Stevens, N.M. 1909: Further studies on the Chromosomes of Coleoptera. *J. Exp. Zool.* , **6:** 101-121.

Strasburgor, E.H. 1936: Uber Storungen der Eientwicklung bei Kreuzungen Von *Epolachna Chrysomelina* F. mit *Epilachna capensis* Thunb : Z.I.A.V., **71:** 538-545.

Suamalainen, E. 1958. On pplyploidy in animals. *proc. Finn. acad. sci. Lett., 1-15pp.*

Sweetman, H. L. 1958. *The principles of biological control.* W. M. Brown Co., Dobuque, Lowa, 452 pp.

Takenouchi, Y. 1955a . Notes on cytology and hybridization in to puzzling species of *Epilachna* (Cooccinellidae- Coleoptera). *Annot. Zool.* Japon. **28:** 238 – 243.

Takenouchi, Y. 1955b. A further chromosomes survey in thirty species of weevils (Curculionidae, Coleoptera). *Jap. J. Zool.,* **11:** 425-441.

Takenouchi, Y. 1968 A modification of the Xyp sex-determining mechanism in a lady-brid beetle, *Epolachna pustulosa* Kono (Coccinellidae: Coleoptera). *Chromosome inform, Service* No. **9:** 25-27.

Tanimoto, C. 1975. Variations of esterase isozyme in *Harmonia axyridis* pallas. *Jpn. J. Appl. entom. Zool.,* **19:** 292-294.

Thompson; Fanning , J. Morris, Clayton, J. Smith, Kenneth, I. 1969. (Res. Div. Agr. Res. Serv., Ithaca, N. Y.) Annual rev. Biochem. **38:** 137-158. : *Chem. abstr,* **71 :** 87496 d.

Timberlake, P. H. 1943. The Coccinellidae of ladybeetles of the Koebele collection – part I. *Hawaii. Plant. Rec.,Honolulu,* 47 (1) : 1-67.

Timofeeff-ressovsky, N. W. 1940. Zur analyse des polymorphismus bei *Adalia bipunctata* L. Biologisches *Zentralblatt,* **60:** 130-137.

Tjio, J. H. and levan, A. 1953. Comparative Idiogram analysis of the rat and Yosida rat sarcosoma. *Hereditas,* **42:** 218-234.

Tsurusaki, N. Nakano S. and Katakura, H. 1993. Karyotypic differentiaion in the phytophagous ladybird beetles Epilachna vigintioctomaculata complex and its possible relevance to the reproductive isolation. with a note on supernumerary Y chromosomes found in *E. pustulosa Zool. Sci.* **10:** 997-1015.

Van Dyk, D.1990. Genetic Varition within the endemic Southern African murid species *otomys unisulcatus.* (Cited from Akbar, K. M.1995. Ph. D. thesis. R.U. Bangladesh.

Verma, P. S. and Agarwal , V. K. 1981. Cytology . *Chand & Co. Ltd.* Delhi, 350 pp.

Vosa, C. G. 1977. Heterochromatic Patterns and species relationship. *The Nucleus.* **20:** 33-41.

Weise, J. 1892. Les Coccinellides du Chota-nagpore. *Ann. Soc. Entomol. Belgique,* **36** : 16-30.

Weise, J. 1895. Insects du Bengale. 36^{e} Memoire. Coccinellidae. *Ann. Soc. Entomol . Belgique,* **39 :** 151-157.

Weismann, A. 1888. Cited from Verma, P. S. and Agarwal , V. K. 1981. Cytology . *Chand & Co. Ltd.* Delhi, 6 pp.

White, M. J. D. 1954. Speciation in Animals. *Australian J. Sci.* **22:** 32-39.

White, M. J. D. (ed.) 1973. The chromosomes. Chapman and Hall, London, 214pp.

Yadav, J.S. and Dange, M.P. 1989.: Chromosomal investigations on eight species of Histerids (Coleoptera: Histeridae) *Elytron,* **3:** 103-111.

Yadav, J.S.,& Pillai, R.K.1974. Chromosome number and sex determining mechanism in 28 species of Indian Coleoptera C.I.S, **16:** 20-22.

Yadav, J.S., Pillai, R.K. and Karamjeet 1979.: Chromosome numbers of Scarabaeidae (Polyphaga: Coleoptera.) *The Coleopterist Bulletin* **33** (3) 309-318.

Yasuda, H. & Dixon, A.F.G. 2002 Sexual Dimorphism in the two spot ladybird beetle *Adalia bipunctata*: develpmemtal mechanism and its consequence of mating. *Ecological Entomology* **27:** 493-498.

Yoshitake, N. and Aruga, H. 1950. Studies on the amino acids in the silkworm 111, on the free amino acids in the blood of larvae. *J. sericult, sci.* **19:** 534-535.

Yosida, T. H. 1944. Chromosome studies in the Coleoptera. I. *Jap. J. Genet.* **20:** 107-115.

Yosida, T. H. 1948. Distinction of three species of *Epilachna based* on the cytogenetical evidence. *Mastumushi.* **2:** 107-109. (In Japanese).

Yosida, T. H. 1949a. Chromosome studies in Coleoptera III. The structure and formative elements of the sex-chromosomes of *Luperodes paraeustua* Motsch., with consideration of their evolution . *Japan. J. Genetics.*, **24:** 156-162.

Yosida, T.H. 1952. Chromosome studies in the Coleoptera, V. Papers *Coord. Comm. Res. Genet.* **3:** 41-49.

APPENDIX

Bangladesh J. Zool. 31(2): ...-219, 2003

KARYOTYPE ANALYSIS OF TWO *EPILACHNA* SPECIES (COLEOPTERA : COCCINELLIDAE)FROM BANGLADESH

RINA DAS[1], KAMRUL ISLAM[2] AND MD. SOHRAB ALI

Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: Two phytophagous ladybird beetles, namely, *Epilachna vigintiocto-punctata* (Fab.) and *E. pusillanima* (Muls) of Coccinellidae were cytologically investigated for karyotypes. They were found to possess 2n = 18 (8 AA + Xy) and 2n = 16 (7AA + Xy) chromosomes, respectively in spermatogonial cells. Detailed comparison of karyotype using principal components analyses revealed a considerable divergence between the two species. In *E. vigintioctopunctata*, 4th and 5th autosomes pairs were submetacentric (sm) and the rest including X chromosomes are metacentric (m). Centromere was not detected in the minute y chromosome. The karyotype formula of the species was (2AA sm + 6AA m + X m + y). The karyotype formula of the species *E. pusillanima* was (5AA sm + 2AAm + X m + y) where 1st, 2nd, 3rd, 4th and 7th autosome pairs were submetacentric and rest were metacentric. The nature of y chromosome was the same as in the former species. The ratio of autosomes ranged from 12.31% to 5.9 % in *E. vigintioctopunctata* while it was13.66 % to 9.65 % in *E. pusillanima*. The ratio of sex- chromosomes was 23.33 % in the former and 20.69 % in the latter. These non-overlapping karyotypic features seemingly characteristics for differentiating the species.

Key words: *Epilachna*, Chromosome, Karyotype.

INTRODUCTION

The genus *Epilachna*, a phytophagous lady bird beetle, belongs to the family Coccinellidae under the order Coleoptera. In this family there are about 4500 species of which one sixth are Epilachninae (Dieke 1947). These beetles of different forms use to cause considerable damage to various cultivated plants especially belonging to the families Cucurbitaceae, Solanaceae and Papilionaceae. The beetles are identified by the taxonomists depending on their morphology. Many species of the genus *Epilachna* show considerable interspecific variation which make them difficult to identify properly. It is a well-known fact that where the morphological features are difficult to measure, cytological informations may play important roles to solve the taxonomic problems. For this reason, several cytological studies on this genus have been carried out by different workers (Stevens 1909, Takenouchi1955, Smith 1956, 1959, 1960; Dasgupta 1963, Sharma *et al.* 1959, Saha 1973, Kacker 1993).

[1]Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh. [2]Department of Genetics and Breeding, University of Rajshahi, Rajshahi-6205, Bangladesh.

Bangladesh has an agrobased economy. The control of pests of agricultural crops is a vital to boost its economy. Therefore, complete karyotype of each species under this genus is necessary for their authentic identification. Unfortunately, there is, so far, no reports are available on the cytology of the species of this genus from Bangladesh. In the present investigation, an attempt was undertaken to analyse the Karyotype of two *Epilachna* species, namely, *E. pusillanima* and *E. vigintioctopunctata*.

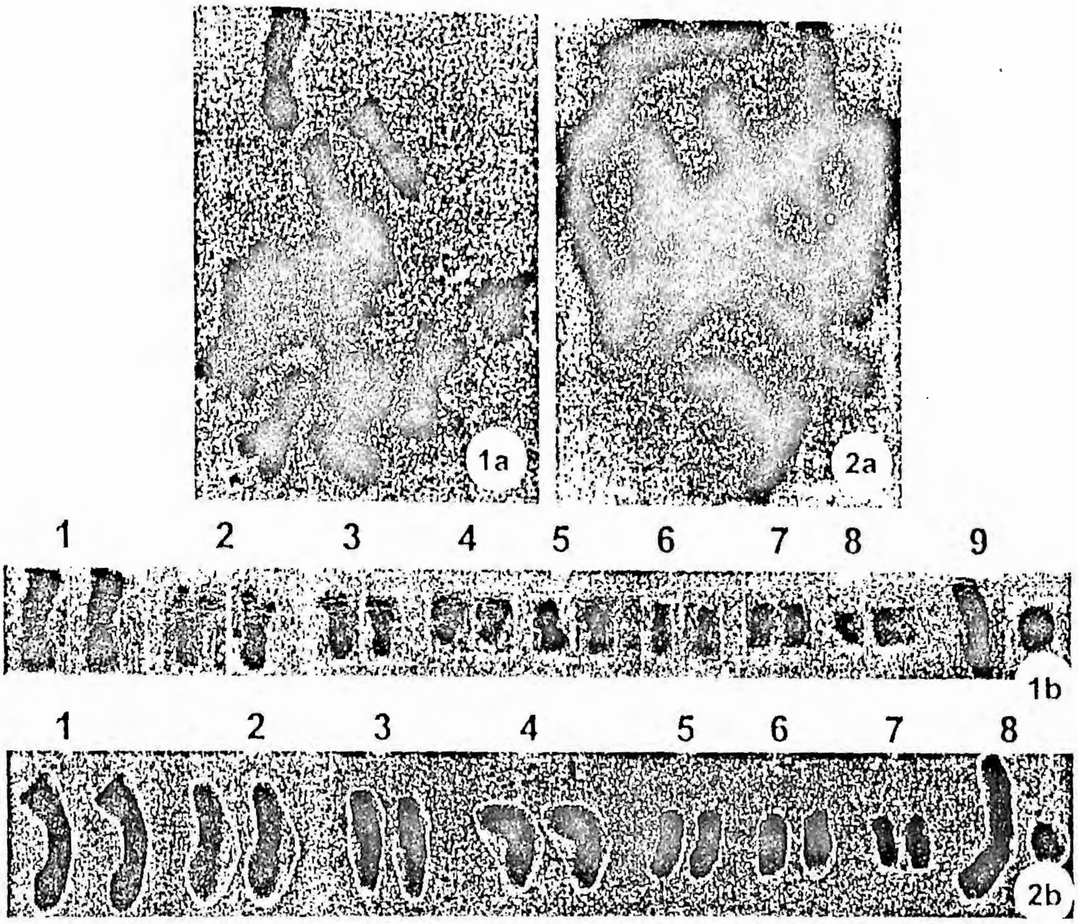
MATERIAL AND METHODS

The adult males of the phytophagous coccinellid beetles, *Epilachna pusillanima* and *E. vigintioctopunctata*, were the two insect species of the present investigation. The insects were collected from plants especially of the family Solanaceae and Cucurbitaceae from the suburban areas of Rajshahi. The males were sacrifced for dissecting out the testes after treated with 1% colchicine solution for 5-6 hrs. The testicular airdrying technique given by Crozier (1968) was used with certain modification suggested by other workers (Mittal *et al.* 1989, Katakura *et al.* 1993). Centromeric formula was derived on the basis of l/s ratios proposed by Leven *et al.* (1964).

RESULTS AND DISCUSSION

Epilachna vigintioctopunctata (Fabricius): The spermatogonial plates revealed the chromosome types 8AA + Xy (Fig. 1, Table 1). The nearer sized chromosomes were considered as autosomes while the other as sex chromosomes X and y. In number, 16 were autosomes and two were sex chromosomes. Of the autosomes, 4th and 5th pairs were submetacentric (sm) while the rest were metacentric (m). The X chromosome was also metacentric and large while y was the smallest and dot shaped. When arranged in the karyotype (Fig. 1a) according to their descending order of size, they exhibit a gradual serriation. The autosomes ranged from 1.95 μm to 4.06 μm (Table 1) in length while the X and y measured 6.2 μm and 1.5 μm, respectively. A calculation of the mean relative lengths of the chromosomes showed that the X was the largest of the complement, it was 0.19 μm. The autosomes (AA) decrease gradually from 0.12 to 0.06 μm The y chromosome measured 0.04 μm (Table 1). X and y chromosomes were found in separate positions.

Epilachna pusillanima (Mulsant): In the spermatogonial plates (Fig. 2, Table 2), 16 chromosomes of which 7 pairs were autosomes and 1 pair was sex chromosome. Of the autosomes 5th and 6th pairs were metacentric (m) and rest were submetacentric (sm). X was also metacentric. The y chromosome was dumble shaped, smallest and 0.98 μm in length, 0.03 relative length (RL). Centromeric position on y chromosome was undetectable. The autosomes varied in lengths from 3.27 μm to 4.59 μm, while X measured 5.97 μm in length (Table 2). Chromosomal formula for the species is 2n = 7 AA (5 sm + 2 m) + X(m) y. The karyotypes are shown in the Fig. 2a.



Figs. 1-2: Showing the metaphase plates and its karyotypes of the two said species.

The characteristic intraspecific polymorphism of colour peculiar in many of Coccinellidae species attracted the attention of population geneticists. The correlation between the phenotypic colour polymorphism and chromosomal polymorphism in this family may be established firstly, with the advancement in techniques for analysing the chromosomes and secondly, with the accumulation of sufficient comparative karyological data.

In Coccinellidae, the number of autosomes ranges from 5 to 11 pairs (Agarwal 1960, Smith 1953, 1960). About 55 species are found to have the 9AA + Xyp type of karyotype. The subfamily Epilachninae, which is supposed to be primitive, has 9AA + Xyp in majority of its species (Yadav and Pillai 1979). The

Table1. Morphometric data for the spermatogonial chromosomes of *E. vigintioctopunctata* (Fab)

Chromosomes /pair	Mean length of short arm s ± S.E. μm	Mean length of long arm l ± S.E. μm	Total length s + lμm	Relative length RL	Centromeric index CI	Arm ratio AR	Centromeric type	Karyotype
1	1.62 ± 0.01	2.44 ± 0.01	4.06	0.12	39.90	1.51	m	2 AA sm +
2	1.60 ± 0.01	2.40 ± 0.01	4.00	0.12	39.98	1.51	m	6AAm +
3	1.45 ± 0.01	2.15 ± 0.01	3.60	0.11	40.28	1.48	m	Xm+y
4	1.14 ± 0.01	2.24 ± 0.01	3.38	0.10	33.73	1.96	sm	
5	1.11 ± 0.01	1.99 ± 0.01	3.10	0.09	35.81	1.79	sm	
6	1.26 ± 0.01	1.44 ± 0.01	2.70	0.08	46.67	1.14	m	
7	1.17 ± 0.01	1.30 ± 0.01	2.47	0.07	47.37	1.17	m	
8	0.94 ± 0.01	1.01 ± 0.01	1.95	0.06	48.28	1.07	m	
X	2.49 ± 0.01	3.71 ± 0.01	6.20	0.19	40.16	1.49	m	
y	1.50 ± 0.01	-	1.50	0.04	-	-	-	

Mean total length of the genome = 32.98 μm; 2n = 18.

Here, $RL = \frac{\text{Length of a particular chromosome (s+ l)}}{\text{Total length of the genome (s + l)}}$; CI = s/s + 1; AR = l/s.

Table 2. Morphometric data for the spermatogonial chromosomes of *E. pusillanima* (Mulsant).

Chromosomes /pair	Mean length of short arms s ± S.E. μm	Mean length of long arm l ± S.E. μm	Total length s + l μm	Relative length RL	Centromeric index CI	Arm ratio AR	Centromeric type	Karyotype
1	1.63 ± 0.01	2.96 ± 0.01	4.59	0.14	39.90	1.81	sm	5 AA sm +
2	1.52 ± 0.01	2.65 ± 0.01	4.17	0.12	39.98	1.74	sm	2AA m +
3	1.45 ± 0.01	2.51 ± 0.01	3.96	0.12	40.28	1.72	sm	Xm + y
4	1.38 ± 0.01	2.49 ± 0.01	3.87	0.11	33.73	1.80	sm	
5	1.30 ± 0.01	2.15 ± 0.01	3.45	0.10	35.81	1.65	m	
6	1.24 ± 0.01	2.11 ± 0.01	3.35	0.09	46.67	1.69	m	
7	1.17 ± 0.01	2.10 ± 0.01	3.27	0.09	47.37	1.79	sm	
X	2.48 ± 0.01	3.49 ± 0.01	5.97	0.18	48.28	1.40	m	
y	0.98 ± 0.01	-	0.98	0.03	40.16	-	-	

Mean total length of the genome = 33.59 μm; 2n = 16.

present report of *E. vigintioctopunctata* and *E. pusillanima* partially apart with the general pattern. The X and y chromosomes are not in Xyp form. Tasurusaki *et al.* (1993) found $2n = 20$ in male beetles of *E. vigintioctopunctata*. In the diploid complement of *E. vigintioctopunctata*, Agarwal(1960) observed 18 chromosomes and the species had XY : XX mechanism of sex determination, the Y approaches the X in size. In the said species 2n =18 (16 AA + X +y) also reported by Yasida (1948) and Bose (1948). The present result agreed with these. Yasida (1948) observed the presence of minute y chromosome. In the present findings y is also minuten. But, Bose (1948) and Agarwal (1960) found the large Y approaching the X in size (16 AA + X +Y) in this species. All the species belonging to the subfamily Epilachninae reported so far have the 18 or 20 chromosomes and an XY : XX mode of sex determination (Stevens 1909, Hoy 1918, Strasburger 1936, Yasida 1944, 1948, Takenouchi 1955).

The Epilachninae have the chromosomes 2n = 10, 18 and 20 in most of the species (Kacker 1993). Yasida (1944, 1948) reported 20 chrosomes (18AA + X + Y) for *E. pustulosa*, *E. vigintioctopunctata*, *E. niponica*. In the species *E. dodecastigma* (Weid) Saha (1973) observed 2n = 14 chromosomes. In the present study 2n = 16 chromosomes were found in the diploid cell of *E. pusillanima*. The same number of chromosomes were found in another species of *Afissa purvulosa* (Crotch) by Kacker(1993).

In the family Coccinellidae, the diploid chromosome number varied from 14 to 20 (Smith 1960) with different types of sex determining mechanism *viz*: unequal X and y, Xyp, and neo XY. In the sub family Epilachninae, out of 5 species worked out, all show 20 chromosomes as diploid number with Xyp type of sex mechanism (Takenouchi 1955, Agarwal 1960, 1961). But, the present study showed the presence of 18 and 16 chromosome as diploid number in the two separate species with unequal X and y. In this point, the chromosome numbers tally with most of the previously studied species but differing in the sex mechanism. Very often it was found that in males of bisexual species of Coccinellidae, a pair of heteropycnotic sex chromosomes usually associated in a typical parachute like manner, conveniently denoted as Xyp. In this findings "Xyp" was undetectable.

If we compare these results with the differences in the chromosomes numbers and sex chromosomes the discrepancy may be well explained either by loss or by fusion of the chromosome.

Akbar (1995) reported X chromosome as the largest and easily recognizable while y is the smallest and not always identifiable. Yasida (1948) observed the presence of minute y chromosome, although Agarwal and Bose (1948) found large Y chromosome. In the present study a small y chromosome was found out which agreed well with the findings of Yasida (1948).

Thus, it may be summarized by saying that the chromosome numbers and sex determining mechanism of most of the species studied varies but centering round the type number 2n = 14 to 20 (Smith 1960).

Acknowledgement: The work was carried out at the Institute of Biological Sciences (IBSc) of the University of Rajshahi. Our thanks are due to the Director, the teachers and all the other stuff of IBSc for providing the facilities.

LITERATURE CITED

AGARWAL. U. 1960 . Chromosome number and sex mechanism in sixteen species of Indian Coleoptera. *Curr. Sci.* **29**: 140-144.

AGARWAL. U. 1961. Studies on the chromosomes of five species of Coccinellidae (Coleoptera). *Cytologia* **26** : 285-293.

AKBAR. K. M. J. U. 1995. Cytogenetical studies of the rats and mice in Bangladesh. Ph. D. Thesis, I.B.Sc. Rajshahi Univ. 204 pp.

BOOTH. R. G. 1993. International Institute of Entomology. Identification services Report 2655/11655 Asia. 2nd February 1993. 2 pp.

BOSE, I. 1948. The association of non homologous chromosomes in the spermatogenesis of the ladybird beetle *Epilachna vigintioctopunctata* (Fab). *Proc. Zool. Soc.Bengal.* **1**: 131-140.

CROZIER. R. H. 1968. An acitic acid dissociation Air-drying techechnique for insect chromosome, with acito-lactic orcein staining. *Stain tech.* **43**: 171.

DASGUPTA. J. 1963. The cytology of *Cybister dimbatus. Gymnopleurus koengii, Aulacophora* and *Alcides* sp. (Insecta: Coleoptera). *Proc. Zool. Soc* . **16**: 123-134.

DIEKE. G. H . 1947. Lady beetles of the genus *Epilachna* (Sens: lat.) in Asia. Europe and Australia, *Smithson Misc. Coll.* **106** : 1-183.

HOY. W. E. 1918. A study of somatic chromosomes. 11. *Biol. Bull.* **35**:166-174.

KACKER. R. K. 1976. Studies on the chromosomes of Indian Coleoptera. 6 chromosome number and sex determining mechanism in 15 species of Coleoptera. *Newsl. Zool. Surv. India.* **2**: 48049.

KACKER. R. K. 1993. Chromosomes and phylogeny of Coleoptera, *Zool. Surv. India.* **147**: 8-9 .

KATAKURA. H., NAKANO. S. and TSURUSAKI. N. 1993. Karyotypic differentiation in the phytophagous ladybird beetles *Epilachna vigintioctomaculata* complex. *Zoologikal science. Japan.* **10**: 997- 1015.

LEVAN. A. K. FREDGA and SANDBERG. A. A. 1964. Nomenclature for centric position on chromosomes . *Hereditas (Lund).* **52**: 201-220.

MITTAL. O.P., GILL. T. K. and DAID. P. 1989. Karyological investigations on five Indian coccinellids (Coleoptera). Perspectives in Cytology and Genetics. **6**: 383-88.

SAHA. A. K. 1973. Chromosomal studies of the Indian Coleoptera. *Cytologia* **38**: 363.

SHARMA, G . P., PRASHAD, R. and PAUL, M. K. 1959. Chromosomal polymorphism in *Coccinella septempunctata* L. (Coccinellidae: Coleoptera) *Res . Bull.* Punjab Univ. (N. S) **10**: 218-219.

SMITH, S. G. 1953. The chromosome number of Coleoptera. *Heredity* **7**: 31-48.

SMITH, S. G. 1956. Chromosomal polymorphism in bank weevil. *Nature* **177**: 386.

SMITH, S. G. 1959. The cytogenetic basis of speciation in Coleoptera. *Proc. Intern Congr.* Genet. 10th Congr. Montreal. *1* : 444-450.

SMITH, S. G. 1960. Chromosome numbers of Coleoptera. II. *Can J. Genet. Cytol.*, **2**: 67-88.

STEVENS, N. M.1909. Further studies on the Chromosomes of Coleoptera. *J. Exp. Zool.*, **6**: 101-121.

STRASBURGOR. E. H. 1936. Uberstorungen der Elentwicklung bei Kreuzungen von *Epilachna chrysomalia* F. mit *Epilachna capensis* Thumb : *Z. I. A. V.*, **71**: 538-545.

TAKENOUCHI, Y. 1955. Notes on cytology and hybridization in two puzzling species of *Epilachna* (Coccinellidae : Coleoptera). *Annot. Zool. Japan.* **28**: 238-243.

TSUIKUSAKI, N., NAKANO S. and KATAKURA, H. 1993. Karyotypic differentiation in the phytophagous ladybird beetles *Epilachna vigintioctomaculata* complex and its possible relevance to the reproductive isolation, with a note on supernumerary Y chromosomes found in *E. pustulosa*. *Zool. Sci.*, **10**: 997-1015.

YADAV, J. S. and PILLAI, R. K. 1979. Evolution of Karyotypes and Phylogenetic Relationships in Scarabaeidae (Coleoptera) *Zool. Anz. Jena*, **202** : 105-118.

YASIDA, T. 1944. Chromosome studies on the Coleoptera, I. A Study of Chromosomes in ten species of Coccinellidae. *Jpn. J. Genet.* **20**: 107-114.

YOSIDA, T. H., 1948. Distinction of three species of *Epilachna based* on the cytogenetical evidence, *Mastumushi.*, **2**: 107-109. (in Japanese).