Review of literature
Chapter II

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Grasshoppers that belong the family Acrididae (Orthoptera) are easily identifiable. While this group of insects contains some dramatic variation, there are a few morphological features that remain fairly consistent. Acridids have 3-segmented tarsi, short ovipositors, tympana found on the sides of the first abdominal segment and the antennae are almost always shorter than the body. Adults of some species are winged, while other species are wingless or have extremely reduced wings. Eggs are usually deposited in soil and in clusters or pods with as many as 100 eggs. Grasshoppers are hemimetabolous insects and therefore go through a gradual metamorphosis. Each instar looks like a smaller version of the adult, with wings not fully formed until adulthood (in the winged species). All grasshoppers are plant feeders, but will occasionally feed on dead insects, leaf litter, or even dung.

The superfamily Acridoidea is important because it includes pest species as well as many unusual and poorly understood insects that cause devastating economic losses. Although phylogeny of this group has been investigated from a variety of perspectives and at different taxonomic levels, phylogenetic relationships within Acridoidea superfamily remain unresolved, for example, it is still lacking in general consensus on the relationships between families (Dirsh, 1975; Liu and Jiang, 2005). Blanchet et al., (2010) using an enriched methodology developed microsatellite markers from two
species, *Calliptamus italicus* and *Calliptamus barbarus*. The polymorphic markers were tested on different populations of *C. italicus*, *C. barbarus*, *C. wattenwylianus*. Two markers were amplified on the three species, as well as four on *C. barbarus* and two on *C. italicus*. In each species, 9 to 23 alleles per locus were observed. Based on the results it was concluded that the molecular markers might prove to be a new and interesting tool for *Calliptamus* population genetics and dispersion studies.

Because of their economic importance, grasshoppers have been the subject of thousands of publications, many with identification keys included. One of the most comprehensive of these was attempted by Otte (1984) in which he developed keys to all the species. However, most identification keys are regional in nature (Blatchley 1920; Capinera and Sechrist, 1982; Richman et al., 1993). Having fresh specimens is helpful because much of the key involves the color of grasshoppers. Grasshopper specimens tend to lose much of their color, with the green colors turning brown after drying and preservation. However, wing color remains fairly distinct, with only slight fading after preservation. While occasionally the abdomen in some species shrivel, is considered relatively unimportant because the abdomen usually is not an important taxonomic feature. The cerci, supra-anal plate and the sub-genital plate, which are very important in identification, are usually unaffected by this shriveling. Many of the melanopline species and some others, are only identifiable based on male genitalia. For this reason, it is very important that
males be collected from each population to associate with the females of the same species.

Females are not always identifiable, so it is important to acquire males and identify females by association. In this key, length, when not specified otherwise, refers to the distance from the front of the head to the tip of the wings in long-winged species. In short-winged species, length refers to the distance from the front of the head to the tip of the abdomen. If the abdomen is shrunken or curved, the tips of the femora can be used instead, as this approximates the abdomen length (Smith et al., 2004).

The relationship between mouthpart structure and diet has been known for years. This connection between mouthpart morphology and specific food types is incredibly pronounced in the class Insecta (Snodgrass, 1935). As insects have evolved and adapted to new food sources, their mouthparts have changed accordingly. This is an extremely important trait for evolutionary biologists (Brues, 1939) as well as systematists (Mulkern, 1967).

On the other hand Mouthpart consistency within subfamilies indicates that evolution is just as important as ecological factors in determining food plants; for most subfamilies there is a strong association with a particular form of vegetation. It is evident that the ability, or tendency, of grasshoppers to change hosts is partly limited by the structure of their mandibles. However, because there are exceptions to the strong association of cyrtacanthacridines with forbs and gomphocerines with grasses, we see evidence that behavioral
plasticity or ecological opportunism is present even in relatively primitive taxa such as Orthoptera.

Due to the clustered organization of multigene families and other repetitive DNAs, they have been regarded as an important source as chromosomal marker for analysis of karyotypic evolution, genomic structure and origin and evolution of B chromosomes in animals. In grasshoppers the mapping of repeated elements is primarily concentrated in analysis of number and location of rDNAs and histone genes and in the lesser extends satellite DNA (satDNAs). Concerning multigene families in Acrididae the major rDNA was mapped in 53 species, the histone genes were located in chromosomes of 39 species and the 5S rDNA distribution was described in about 30 species. Further, findings of Oliveira et al (2011) significantly contribute to understanding the organization/evolution of multigene families in the insect genomes.

An insect’s habitat is the area of the environment that provides the resource requirements for a discrete phase of its life. Friauf (1953) noted that classification of orthopteran populations in relation to habitats has been a difficult problem, though it is apparent that assemblages of grasshoppers will vary in density and species composition in relation to differences in vegetation, soil, temperature and humidity of the habitat. Friauf (1953) found it most satisfactory to associate orthopteran fauna with habitat classification based on the dominant flora.
Recent estimates (Kevan 1982; Günther, 1980, 1992; Otte 1994-1995) indicate some 2400 valid Caeliferan genera and about 11000 valid species described to date. Many undescribed species exist, especially in tropical wet forests. Caelifera are predominantly tropical, but most of the superfamilies are represented worldwide. By insect standards the Caelifera are relatively homogenous; all have jumping back legs and are almost exclusively herbivorous. None the less they show considerable diversity.

As adults they range in size from a few millimeters to more than 15 cm in length, are flight or flightless, occupy virtually all non-marine habitats in which plants can live (including deserts, water surfaces, the crowns of forest trees, grasslands, or underground); they eat algae, mosses, the leaves and reproductive organs of ferns, gymnosperms and angiosperms, or even the roots of the latter, with all degrees of food plant specialization from wide-range polyphagy to strict monophagy.

Caelifera are probably the oldest living group of chewing folivorous insects. Tettigoniioidea may predate them geologically, but it is unclear how herbivorous these were, as many Tettigonioids are even now still carnivorous or omnivorous. The fossil Caelifera are reviewed by Zeuner (1941-1944), Sharov (1971), Kukalova-Peck (1991), Carpenter (1992) and Ross and Jarzembowski (1993). The split between the Caelifera and the Ensifera is not more recent than the Permo-Triassic boundary (Zeuner 1939). The earliest known representatives of an extant Caeliferan Superfamily are the extinct *Regiatidae (Tridactyloidea) from the Lower Jurassic (Gorochov 1995).
Essentially modern Eumastacids are known from the mid-Jurassic, modern Tridactyloids and Tetrigoids from the early Cretaceous, Acrifolds from the Eocene. Most, possibly all, of the modern super families probably developed in the Jurassic.

Among other synapomorphies the Caelifera is distinguished from the Ensifera by the structure of the ovipositor, in which the original 6 valves are reduced to 4 functional ones with transverse musculature, by antennae composed of less than 30 segments and by the absence of auditory organs on the prothorax - if a tympanum or other hearing organ is present, it is abdominal. The sperm are thin and elongate, with an acrosome inserted on the nucleus by means of two lateral processes.

Due to the virtual absence of explicit schemes in the modern literature, this hypothesis follows no particular publication, but it is shared by most modern morphologists (Rentz, 1991). As in several of the insect orders, few formal phylogenetic analyses of the Caelifera have been undertaken. The major sources of phylogenetic opinion lie in the discussion sections of primarily taxonomic or morphological publications and are rarely supported by explicit data. Cladistic analysis of morphological data matrices is rare. Recently molecular data been used to examine phylogenetic relationships within this group.

One category of phylogenies is derived from wing venation, as exemplified by the schemes of Zeuner, Sharov, Ragge and Gorochov. These works, however, the emphasis is on the Ensifera, which have a much more
extensive fossil record and the Caelifera are not treated in detail. Further, some of the Caeliferan relationships implicit in the scheme proposed by Sharov are not supported by any obvious evidence (Amédégna, 1993). A further problem lies in the identification of the plesiomorphic form of the venation. For example, Ragge concluded that the Pneumorids were the most primitive Caeliferans on the basis of the similarity of their venation with that of the Palaeozoic *Palaeodictyoptera.

However, it is not considered to be ancestral to the orthopteroid insects, their significance in this respect is dubious. A second category of phylogeny is derived primarily from the male internal genitalia. The most rigorous analysis of genital characters for large numbers of taxa (Amédégna, 1977) was based on phenetic similarity rather than cladistic methodology and the original data matrix is not available. The resultant phylogenies are however generally in agreement with those derived from molecular data. Numerical phenetic analysis has also been applied to non-genital morphological characteristics to produce a phylogeny of the Orthopteroid orders (Blackith and Blackith, 1967), but the number of Caelifera included in the sample was very small.

Molecular approaches to higher level Caeliferan systematics consist to date of a) cladistic analysis of allozyme polymorphism of glycolytic enzymes (Colgan, 1989), again using a very small taxonomic sample and b) parsimony and distance analysis of a much larger sample of mitochondrial and nuclear ribosomal sequences by the present authors (Flook and Rowell, 1997-2000).
The topologies differ in the levels at which they resolve, but the branching order of the Super families is not contradicted in either phylogeny. The mitochondrial data do not resolve well the oldest branches, presumably due to site saturation, whereas the 18S data do not resolve well the youngest branches, due to insufficient divergence. For these reason combinations of these data sets, with appropriate precautions, was used to achieve a broader resolution. This strategy has now allowed us to resolve the phylogeny of the entire Caelifera at the level of Superfamily.

These molecular data often support unequivocally one of several schemes previously proposed on morphological grounds. Thus, for example, the basal placing of the Tridactyloids, the relatively close relationship of the Tetrigoids and Eumastacoids and the placement of the Trigonopterygoids as a separate group between the Eumastacoids and Pneumoroids, have all been previously suggested by some morphologists and will probably be generally accepted without dissent.

Among original findings is the dismemberment of the Pneumoroidea sensu Dirsh, with the resultant superfamily status of the Tanaoceroidea, the placement of the Xyronotidae as the sister family of the Trigonopterygidae and their joint elevation to superfamily status as the Trigonopterygoidea and the restriction of the Pneumoroidea sensu novo to the sole family Pneumoridae. These results are supported by very high levels of statistical probability. The data do not as yet allow resolution of the branching order of the Tanaeceroidea and Trigonopterygoidea.
The other important finding concerns the Pamphagoidea sensu Dirsh. Some morphologists have seen this group as part of the Acridoidea, others considered the differences in genitalia to warrant separate superfamily status; it was however generally accepted that a) they are monophyletic or at least closely related and b) they are primitive with respect to the remaining Acridoidea. The ribosomal sequences however, indicate that this group is actually polyphyletic; the Pyrgomorphidae is indeed a monophyletic clade branching off well before the Acridoidea, but the remaining taxa (Pamphagidae and Lentulidae) are embedded within Acridoidea.

The study raised Pyrgomorphidae to superfamily status, dropping the superfamily Pamphagoidea and redefining the Acridoidea s.n. to include the Pamphagidae and Lentulidae. The allozyme data of Colgan (1989), which included a pyrgomorphid, are compatible with our proposition, but do not confirm it, as no other pamphagoid sensu Dirsh was included. Recently, however, Eades (2000) has offered new interpretations of the phallic anatomy of pyrgomorphids, pamphagids and acridids which support the new classification. The age of the Caelifera and their multiple convergences to common habitats make their morphological classification difficult below the level of the superfamily. For the past 50 years most weight has been placed on the internal genitalia, especially those of the male sex. These are effectively never available in fossil material: the palaeontological classifications, as in most insect orders, are founded principally on wing venation (Kukalova-Peck, 1991).
Modern authors have usually divided the living Caelifera on morphological grounds into 7 higher taxa, which we here treat as superfamilies. The majority of living Caelifera belongs to the family Acrididae of the Acridoidea. The difficulties in Orthopteran taxonomy have been discussed by Rentz (1991, p. 378) who remarks: "There are many disparate classifications of the orthopteroid insects (or positions thereof) at the present time. The overriding theme is the escalation of the rank of categories above the tribal level. The lack of congruence among authors contributes to considerable instability."

Dirsh (1975) for the extreme of these views and Kevan (1982) for a synthesis of the classifications." The most disputed superfamilies have traditionally been the Pamphagoidea, which some authorities regard as insufficiently separated from the Acridoidea to merit independent status and the Eumastacoidea, which may or may not include the family Proscopiidae. The position of these taxa is discussed in the Discussion of Phylogenetic Relationships and the accessory page 'A Classification of the Caelifera' provides a proposal for a classification down to the subfamily level which takes in account the results of recent morphological and molecular analyses.

Additionally, there are two Permian/Mesozoic fossil caeliferan taxa, the *Locustopseidae and the *Locustavidae, each sometimes given Superfamily rank, but by others (Gorochov, 1995) grouped as Superfamily *Locustopsoidea. These families are defined only on the basis of wing venation (trifurcate MA and a branched MP+CuA1 in the forewing, branched
MA in the hindwing). At least the former family may be simply an assemblage of primitive forms and contain the early representatives of several of the modern groups.

Molecular phylogenetic analysis of intercontinental taxa above species level provides opportunity to investigate the evolutionary impact of geological and climatological processes in the distant past. Such studies has steadily increased for several insect orders, for instance Coleoptera (Pearson and Vogler, 2001; Davis et al., 2002), Diptera (Barrio and Ayala, 1997; Martin et al., 2002), Hemiptera (Buckley et al., 2002; von Dohlen et al., 2006), Hymenoptera (Leys et al., 2002; Kawakita et al., 2004), Lepidoptera (Zakharov et al., 2004; Hundsdoefer et al., 2005). On the other hand, few have challenged the traditional views on place and time of origin and directionality of migration (Pearson and Vogler, 2001; Costa et al., 2003, Hundsdoefer et al., 2005; von Dohlen et al., 2006).

However, within the Orthoptera, there have been few comparable investigations. In one recent example, a mitochondrial DNA (mtDNA) phylogenetic analysis (Lovejoy et al., 2005) of Old and New World Schistocerca species demonstrated that the genus originated in Africa and not the Western Hemisphere, as had been proposed in an earlier morphological investigation (Song, 2004). Rather, a single east-west, trans-Atlantic dispersal event took place, eventually leading to the establishment of the numerous species presently distributed throughout the Americas.
Rowell and Flook (2004), using mtDNA, speculated on the place of origin of the neotropical subfamily Proctabolinae, identifying proto-Central and South American land areas as alternative locations. Recent findings (Chapco et al., 2001; Amédégnato et al., 2003) challenged the prevailing view (Vickery, 1989) that the subfamily Melanoplinae originated in Laurasia and that during the Pliopleistocene Great Interchange, incursions from the north led to the establishment of taxa in South America. Instead, molecular phylogenetic analyses of mitochondrial genes showed the reverse that the subfamily originated in South America probably in the Early Cenozoic and subsequently, via island-hopping, progressed to establish the Holarctic fauna.

A similar analysis (Contreras and Chapco, 2006) of Holarctic Gomphocerinae supported Vickery’s (1989) contention that there were at least three dispersal events from Eurasia to North America. More recently, with the inclusion of taxa from the southern hemisphere, preliminary analyses have not contradicted that conclusion; however, it has been suggested that the possibility that the subfamily originated still earlier in Gondwanaland.

Another subfamily, whose distribution surpasses even those of Gomphocerinae and Melanoplinae, is Oedipodinae (= Locustinae), or the band-winged grasshoppers. Its over 900 species and 185 genera occur throughout the major continents, making the subfamily the most cosmopolitan among the 30 subfamilies of the Acrididae (Vickery and Kevan 1985, Otte 1995). Among their numbers can be counted several infamous pests, such as the migratory locust (*Locusta migratoria*), the Australian plague locust
(Chortoicetes terminifera) and the clear-winged grasshopper (Camnula pellucida).

The Acrididae belongs to the superfamily Acridoidea of Orthoptera and is the largest family in the Acridoidea. So far, more than 800 species of Acrididae have been described in China (Xia, 1994) and Chinese taxonomists have widely adopted Xia’s taxonomic system of Acridoidea. In this system, Acridoidea was divided into eight families: Pamphagidae, Chrotogonidae, Pyrgomorphidae, Catantopidae, Oedipodidae, Arcypteridae, Gomphoceridae and Acrididae (Zheng, 1993; Xia, 1994).

Taxonomically, oedipodine grasshoppers over the years have been grouped as a tribe, as a subfamily and at times, as a family (Guliaeva et al., 2005). The subfamily designation is now generally accepted (Otte 1984), but not by all (Rentz 1996). In the most recent version of the Orthoptera Species File (OSF2) (Otte, 1994a; 1994b; 1995a; 1995b; Eades et al., 2007; Eades and Otte, 2009), Oedipodinae is subdivided into 15 tribes, of which a few such as Locustini and Sphingonotini occur on two or more continents. Most however, are restricted to only one land mass.

In OSF, the superfamily Pyrgomorphoidea includes only one family Pyrgomorphidae, and the superfamily Acridoidea was divided into 11 families, including the Acrididae, Charilaidae, Dericorythidae, Lathiceridae, Lentulidae, Lithidiidae, Ommexechidae, Pamphagidae, Pyrgacrididae, Romaleidae and Tristiridae. The members of the Catantopidae, Oedipodidae, Arcypteridae, Gomphoceridae and Acrididae in Xia’s system belong to the
same family as the Acrididae in the international system (Kevan, 1982; Flook and Rowell, 1997; Eades, 2007; Xia, 1994). In addition, it had been proven by 18S rDNA (Liu and Jiang, 2005) and 16S rDNA (Liu et al, 2005; Sun et al, 2010) that Catantopidae, Arcypteridae, Gomphoceridae and Acrididae were non-monophyletic. At the molecular level, Liu and Jiang (2005) proposed that the above five families should be grouped into the family Acrididae in accordance with the international system.

Morphological similarities among continentally separated taxa have led to speculations about the subfamily’s historical origins, a topic of interest to orthopterists for about half a century, starting with Rehn’s (1958) seminal paper on North American species. Rehn made brief reference to connections with Eurasian taxa, but on the whole focused on identifying probable centers of origin in the New World. Vickery (1987, 1989) proposed that initially the subfamily had evolved over 100 Mya, before the complete sundering of Pangaea; subsequently, diversification continued in separate Nearctic, Palearctic and Ethiopian centers.

Vickery (1989) also viewed some Nearctic elements as descendants of more recent invaders from the Old World, entering North America via one of several land bridges that had connected the two land masses. The subfamily is poorly represented in the Neotropics (about seven genera), which would suggest that the incursion from the north was fairly recent (Rehn, 1958; Carbonell, 1977).
The subfamily is well represented on the African continent (Otte, 1984), but apart from Vickery’s brief statement cited above, very little (Ritchie, 1981; 1982) has been proposed on the origin of that continent’s oedipodinid fauna. Unfortunately, the few published phylogenies — both morphologically based (Otte, 1984) and molecularly based (Chapco et al., 1997; Rowell and Flook, 2004; Guliaeva et al., 2005; Lu and Huang, 2006) - are inadequate for testing these biogeographic hypotheses. Trees appear either somewhat arbitrary in their construct (Otte, 1984), or they include too few oedipodinids (Rowell and Flook, 2004; Guliaeva et al., 2005), or they focus on only one continent (Chapco et al., 1997; Lu and Huang 2006).

Since recorded time, grasshoppers mainly locusts kindle anthropogenic interest due to their destruction potential of crops, pastures and rangelands resulting food shortages besides their usage as model organism for many scientific inquiry. The literature survey reflects that significant research has been conducted since Uvarov (1966; 1977) to Chapman and Joern (1990) and Gangwere et al. (1997), covering taxonomy and systematics (Kirby, 1914; Dirsh, 1961; 1975; Muralirangan et al., 1993; Vickery, 1997; Storozhenko, 1997), ecology (Lockwood, 1997a) and population dynamics (Lockwood, 1997b), feeding behaviour and host plant association (Muralirangan et al., 1997; Chapman and Sword, 1997), biological control (Lockwood and Ewen, 1997) and molecular evolutionary genetics (Chapco, 1997).

Similarly, fossil Acrididae are known from the Oligocene, Miocene and Eocene (Storozhenko, 1997). Starting from Kirby (1914) and Uvarov
several publications have been made on the description and
distribution of Indian Acridids (Usman and Puttarudhraiah, 1955; Dirsh, 1961
and 1975; Bhowmik, 1985; 1986; COPR, 1982; Prasad Kumar and
Viraktamath, 1991; Muralirangan et al., 1993). The hemimetabolan order
Orthoptera includes twelve superfamilies, of which the superfamily Acridoidea
further consists ten families and its family Acrididae is further classified in to
thirty five subfamilies (Vickery, 1997). The superfamily Pygromorphoidea
comprises the only family Pyrgomorphidae.

Southwood (1961) was undoubtedly the first to consider insect
herbivore richness and its variation among host plant species. Followed by
this and Mac Arthur and Wilson (1967), Strong et al. (1984) brought out this
leading theme having gathered evidences on the diversity of herbivores insects
associated with various host plants to allow inferences on the role of various
causative processes. Lewinsohn et al. (2005) have reported new insights into
stratification and host specialization of herbivores and proposed methods of
community phylogenetic analysis complex networks, spatial and among-host
diversity partitioning and metacommunity models represent promising
approaches for future work.

Studies on the host-plant association of herbivorous insects have
played a central role in our understanding of their ecobehavioural dynamics
and evolution of ecological specialization and this ecological divergence is
facilitated by genetic differentiation or vice-versa. Ecological studies of
grasshoppers are essential to gather enough objective information for
taxonomical revision and relationship. It no longer makes sense in the field of acridology to publish taxonomical lists without the necessary basic environmental species distribution data (Almeida and Camara, 2008).

A vast majority of herbivorous insects restrict their diets to closely related group of plant species and are sometimes monophagous on single species (Bernays and Chapman, 1994; Bernays, 1998). The intimate relationships between specialist herbivores and their host plants in feeding, oviposition, mate finding and predator avoidance make their populations particularly susceptible disruptive selection following shifts to novel host plants (Berlocher and Feder, 2002; Funk et al., 2002).

The lack of adequate species-level phylogenies is often limiting, especially in highly diverse tropical plant group. Analyses of host specificity have mostly approximated phylogenetic relationships between host-plant species by their supraspecific taxonomic ranks that, however, are not commensurate across plant lineages (Losos, 1996). New phylogenetic measures of host specificity and breadth (Symons and Beccaloni, 1999; Webb et al., 2002) have not yet been widely applied. Phylogenetic constraints on host-plant selection may be also examined as a relationship of species turnover between herbivore communities and the phylogenetic distance of their host-plant species (Novotny et al., 2002).

The Lithoscirate acridids are oligophagous or even monophagous, usually feeding the same or closely related plants. The different genera, on the other hand, sometimes eat very different plants and a similar diversity of food
plants is present between species of a few genera. This suggests that past food-plant shifts may have been associated with taxon generation. Sword et al., (2005) observed two genetically distinct host plant-associated lineages in between *H. viridis* grasshoppers feeding on either *G. sarothrae* or *S. mollis* and indicated that evolutionary consequences of host plant-associated divergence are consistent across phytophagous insect groups. Muralirangan et al., (1993) have reported distributional pattern, abundance of sixty Indian acridid species and discussed their eco-behavioural responses.

**Molecular Phylogeny based on Mitochondrial Gene Sequences**

Probably the most commonly used molecule in phylogenetic analyses is mitochondrial DNA. Mitochondrial DNA sequence information are gaining increasing popularity in phylogeneic analyses because of their better resolution. With more than 15,000bp of nucleotide data and 37 genes, mitochondrial genomes are one of the most information-rich markers in phylogenetics (Fenn et al., 2008; Li et al., 2009). The cytochrome oxidase I gene sequence of the mitochondrial DNA has proven to be useful for not only identifying species but also inferring intraspecific variation and phylogenetic relationships in wide array of animal groups, nematodes Kuwata et al., 2006), crab (Kamaruzzaman et al., 2011), mites (Li et al., 2010), Sea-bird lice (Page et al., 2004), gastropods (Remigo and Hebert, 2003), birds (Dong et al., 2010; Lei et al., 2010), fishes (Heras et al., 2009). The phylogenetic utility of mitochondrial genomes has been studied rigorously in the past years,
especially for insects and related groups (Nakamine and Takeda, 2008; Cameron et al., 2007; Lee et al. 2005; Shufran et al., 2000).

Mitochondrial DNA evolves much faster than nuclear DNA, in fact, that mtDNA sequence variation has been found not just between closely related species and this makes them more suitable for phylogenetic analysis of closely related taxa. Nucleotide substitutions between bases belonging to the same family (transitions) occur more frequently than between bases from different families (transversions), reducing the data to purines and pyrimidines, efficiently reduces saturation (Delsuc et al., 2005). Aikawa et al. (2003) constructed phylogenetic trees for four rice grasshopper species belonging to the genus *Oxya* using nuclear ITS2 amd mitochondrial COI sequences and the results were consistent with the conventional grouping based on morphology and reproductive compatibility.

Zhang et al. (2005) analysed phylogenetic relationships of some genera of Pamphagidae based on the mitochondrial 16S ribosomal DNA partial sequences and established monophyletic relationship within the Pamphagidae taxa separated from the outgroup taxa of Pyrgomorphidae and Acrididae. Lovejoy et al. (2006) presented a mitochondrial DNA phylogeny of the migratory grasshopper *Schistocerca* species that supports the monophyly of New World species and their analysis indicates a single trans-Atlantic flight from Africa to South America, followed by extensive speciation and ecological divergence in the New World.
Hong Yin et al. (2003) analysed molecular phylogeny of the acridoidea insects based on 16S rDNA sequence variation. Babcock and Heraty (2000) unambiguously distinguished Encarsia formosa and E. luteola with the sequence variation in the D2 expansion of 28S rDNA. Phylogenetic analyses of the genus Encarsia suggest the D2 region of 28S rRNA gene to be most suitable not only for inferring relationship, but also for taxonomic and diagnostic purposes at species level (Schmidt et al., 2006). Cabrero et al. (2009) analysed chromosome location of H3 and H4 histone gene clusters by fluorescence in-situ hybridisation (FISH) in 35 species of Acrididae grasshoppers belonging to seven subfamilies. Molecular data of 16S rRNA and Cyt B suggest that the taxonomic status of the subfamily Oedipodinae itself is uncertain, given the relationships among its members and a species from another subfamily (Chapco et al., 1997).

Kjer (2004) observed that based on 18S sequence data, there was virtually no support for relationship among orthopteroid orders. Ren et al. (2004) inferred that the nine Oxya species form four well-supported clades and found monophyletic and paraphyletic phylogenetic relationships within those species analysed based on DNA sequences of the mitochondrial cytochrome b gene. Phylogenetic analyses for the molecular data using maximum parsimony and neighbor-joining methods showed that the nine Oxya species form four well-supported clades, which include (1) O. intricata and O. flavefemura; (2) O. japonica and O. bicingula; (3) O. agavisa; and (4) O. chinensis, O. brachyptera, O. adentata, and O. hainanensis, respectively.
In particular, the monophyly of *O. hainanensis* and *O. agavisa* was strongly supported, respectively. However, *O. flavefemura* and *O. intricata*, *O. bicingula*, and *O. japonica* form paraphyletic groups, respectively, and *O. chinensis*, *O. adentata*, and *O. brachyptera* form a polyphyletic group, suggest that they should be merged as few as three species.

The Chinese species of the subfamilies Porthetinae and Akicerinae were distinctly separated from the African taxa. And also the phylogenetic relationships of the eight genera of the subfamily Prionotropsinae were also not clearly resolved (Zhang *et al.*, 2005). A molecular phylogeny of the family Mycetophilidae based on the 18S, 28S and the mitochondrial 16S rRNA is presented by Rindal *et al.* (2009). Hou *et al.* (2007) examined the phylogenetic relationships among the amphipod crustacean genus *Gammarus* species using molecular sequence data from partial segments of the mitochondrial gene 16S rRNA and cytochrome c oxidase subunit I, as well as nearly complete 18S and 28S sequences.

Bayesian analysis of the molecular data revealed monophyletic relationships among the taxa of three subfamilies. Within the subfamily Gnoristinae, the genera *Coelosia*, *Boletina*, *Gnoristae* group with the genus *Docosia*, usually considered being members of the subfamily Leiinae and also no support was found for the subfamilies Sciophilinae and Leiinae. Michez *et al.* (2009) observed that two genera Afrodasypoda and Promelitta of the bee family Melittidae were transferred from the subfamily Dasypodainae to Melittinae based on molecular data.
Degnan *et al.* (2009) suggest a consensus method for using combined multiple loci sequence information to infer the species-tree topology, even when it is discordant with the most likely gene tree. Liu *et al.* (2008) reconstructed phylogeny of Acrididae using 12S rDNA and 16s rDNA and detected monophyletic and non-monophyletic origin of Acridids at subfamily level. Comparisons of the DNA sequences of metazoa show an excess of transitional over transversional substitutions. Part of this bias is due to the relatively high rate of mutation of methylated cytosines to thymine. Postmutation processes also introduce a bias, particularly selection of codon-usage bias in coding regions (Keller *et al.*, 2007).

**Phylogenetic Analysis and Bioinformatics Tools**

We live in the age of comparative genomics and it may seem that there is not much point in reconstructing phylogenies using morphological data anymore and it seems possible that in the not-too-distant future we will be able to have a perfectly accurate and well-supported phylogeny of most living species on earth using molecular data alone (Wiens, 2004). Molecular phylogenetic uses DNA sequence data with an evolutionary perspective for reconstruction of evolutionary relationships for both genes and species. The availability of DNA sequence information in many organisms provides a database to determine the phylogenetic relationships between species or other taxa that were not clear from other traits.

A central principle in all phylogenetic reconstructions is the idea of phylogenetic tree that graphically describes the relationship among different
species. There are two different approaches to determine phylogenetic relationships among organisms using molecular data. The first approach is based on statistical principles that analyse sequence data on the basis of their overall similarity to each other. The second, Parsimony-Based approaches group organisms in ways that minimize the number of substitutions that must have occurred since they last shared a common ancestor and this is the most useful in molecular evolution studies.

Phylogenetic and sequence alignment are closely related fields due to the shared necessity of evaluating sequence relatedness. Sequence alignment is extensively used in the construction of phylogenetic trees, which are used to infer the evolutionary relationships between homologous genes represented in the genome of divergent species. There were a number of programs available on-line to infer phylogenetics from the available sequence data (PAUP, PHYLIP, and MEGA). The MEGA software aims to serve the purposes of phylogenetic inference by using computational and statistical methods with the best suited algorithms and it provides many convenient facilities for the assembly of sequence data sets from files or web-based repositories and it also includes tools for visual presentation of the results obtained in the form of interactive phylogenetic trees and evolutionary distance matrices (Kumar et al., 2008).

Saitou and Nei (1987) introduced the Neighbor-Joining (NJ) method and it has become the most widely used method for building phylogenetic trees from distances. The neighbour-joining method is a greedy algorithm,
which attempts to minimize the sum of all branch-lengths on the constructed phylogenetic tree. Conceptually, it starts out with a star-formed tree where each leaf corresponds to a species and iteratively picks two nodes adjacent to the root and joins them by inserting a new node between the root and the two selected nodes (Mailund et al., 2006).

The minimum-evolution (ME) method of phylogenetic inference is based on the assumptions that the tree with the smallest sum of branch length estimates and this tree would be somewhat different from the neighbour-joining tree, but there would not be statistically significant difference between them (Rzhetsky and Nei, 1993). Maximum parsimony is a character based method that infers a phylogenetic tree by minimizing the total number of evolutionary steps required to explain a given set of data, or in other words by minimizing the total tree length. The maximum parsimony method searches all possible tree topologies for the optimal by using only parsimony informative sites. Phylogenetic tree construction by maximum parsimony from genetic variation data is a fundamental problem in computational genetics with many practical applications in population genetics, whole genome analysis and the search for genetic predictors of disease (Sridhar et al., 2007).

Sandhu et al. (2008) have proposed enhanced small parsimony algorithm to give better score based on number of evolutionary changes needed to produce the observed sequence changes and also give the ancestor of the given sequence data. The overall accuracy of a phylogenetic tree is
often measured as the number of correct taxon bipartitions found on the estimated tree divided by the total number of taxon bipartitions possible for taxa (Bruce et al., 1998).

After the analysis of the 12 combined mitochondrial and nuclear datasets in seven orders of insects using both equal weights parsimony to evaluate phylogenetic utility and Bayesian methods to investigate nucleotide substitution patterns, Lin and Danforth (2004) argue that insect molecular systematists should increasingly focus on nuclear rather than mitochondrial gene datasets because nuclear genes do not suffer from the same substitution biases that characterize mitochondrial genes.

Schmidt et al. (2006) investigated the potential of three different 28S rRNA gene regions, the two expansion segments of the large ribosomal subunit 28S-D2 and 28S-D3 and the first internal transcribed spacer (ITS-1) for phylogenetic applications and found that the D2 region to be most suitable not only for inferring relationships, but also for taxonomic and diagnostic purposes at species level; as the D3 region is the most conserved and ITS-1 the most variable.

It is now commonplace for studies of molecular biogeography to employ a diverse suite of genetic markers, including loci both in the mitochondrial genome (mtDNA) and throughout the nuclear genome (nuDNA). This variety of genetic information is, in many cases, now complemented with broad taxon sampling encompassing a large geographic scope. In most studies that employ a diverse array of genetic markers and a
robust sampling effort, the patterns observed between different genetic marker

types generally align (Avise, 1994). This is true for comparisons between

species as well as phylogeographic structure that arises within species – the

localities that harbour deep splits between mtDNA clades also have

corresponding differences in the nuclear genome (Zink and Barrowclough,

2008).

This observation is one reason the ‘barcoding of life’ project has

proved successful: clades identified in mtDNA are generally concordant with

other phenotypic and genetic information (Kerr et al., 2007). However,

cordant patterns between mtDNA and nuclear DNA are not always

observed (Funk and Omland, 2003; Chan and Levin, 2005). In fact, the

umber of studies that report discordant patterns between mtDNA and nuclear

arkers, while not large, is increasing, especially within the last decade, as

ore researchers have been able to use both types of markers in combination.

In Chinese system of classification, Acridoidea is divided into eight

ilies: Pamphagidae, Chrotonidae, Pyrgomorphidae, Catantopidae,

epididae, Arcypteridae, Gomphoceridae and Acrididae (Zheng, 1993; Xia,

994). However, this system is very different from the international

taxonomic system, the Orthoptera Species File (OSF), in which the

uperfamily Pyrgomorphoidea includes only one family Pyrgomorphidae, and

uperfamily Acridoidea is divided into 11 families, including the

rididae, Charilaidae, Dericorythidae, Lathiceridae, Lentulidae, Lithidiidae,

maxeschidae, Pamphagidae, Pyrgacrididae, Romaleidae and Tristiridae.
The members of the Catantopidae, Oedipodidae, Arcypteridae, Gomphoceridae and Acrididae in Xia’s system belong to the same family as the Acrididae in the international system (Kevan, 1982; Flook and Rowell, 1997; Eades, 2007; Xia, 1994).

**Grasshopper systematics - Molecular phylogeny**

Reconstruction of the evolutionary history of genes and species is currently one of the most important subjects in molecular evolution. If reliable phylogenies are produced, they will shed light on the sequence of evolutionary events that generated the present day diversity of genes and species and help us to understand the mechanisms of evolution as well as the history of organisms. There are numerous methods for constructing phylogenetic trees from molecular data (Nei and Kumar 2000). They can be classified into Distance methods, Parsimony methods, and Likelihood methods. These methods are explained in Swofford et al. (1996), Li (1997), Page and Holmes (1998), and Nei and Kumar (2000).

**Molecular markers and evolution**

Recent advances in molecular biology have provided new approaches to different fields such as population genetics, taxonomy, phylogeny, and evolution (Avise, 1994). It is important to understand how the biology of a species affects its geographic population structure. Genetic data obtained from molecular techniques allows us to infer geographic structure by estimating genetic similarities and population subdivision among populations or examining relationships among genotypes from several populations relative to
this geographic location (phylogeography). Because some insect species have evolved unique or unusual features, they are ideal models for determining the broader roles of geographic structure in evolution (Roderick, 1996).

Among molecular techniques, mitochondrial DNA (mtDNA) analysis has been extensively used because this marker exhibits several advantages. This macromolecule evolves faster than most nuclear sequences and lacks recombination (Avise, 1991; 1994). Because of these properties, it has been extensively used for analyzing phylogeography and population structure in insects (Chapco et al. 1992, Marchant and Shaw 1993, Rossi et al. 1996 and Lunt et al. 1998). Therefore, the analysis of this essentially neutral and rapidly evolving marker in *D. elongatus* could improve our knowledge of the relative importance of different evolutionary forces on chromosomal and isoenzymal distribution.

**Markers for phylogenetic studies - rDNA**

Phylogenetic analysis is regarded as being an intimidating, complex process that requires expertise and years of experience. In fact, it is a fairly straightforward process that can be learned quickly and applied effectively to produce a phylogenetic tree from molecular data for novices. DNA markers such as mtDNA, RAPD, AFLP, microsatellites and ESTs have been used as popular marker systems in insect genetics research. Although there are inherent advantages and disadvantages associated with each marker systems, the choice of applying them depends upon the objectives of a study. Ribosomal DNA is arranged in tandem arrays (containing transcriptional units
coding for three of the four rRNA types) located at one or more chromosome regions, the so-called nucleolus organizer regions (NORs). NORs are evolutionarily dynamic, which makes them excellent markers for phylogenetic studies (Cabrero and Camacho, 1986). Interspecies comparison of NOR number and chromosome location may be a good tool for macroevolutionary studies. Intraspecific variation, moreover, when detected, is indicative of the complex microevolutionary patterns shown by rDNA.

**Ribosomal RNA genes in grasshoppers**

Contreras and Chapco (2006) analyzed molecular phylogenetic evidence for multiple dispersal events in the gomphocerine grasshoppers and the subfamily is provisionally accepted as monophyletic based on portions of four mitochondrial genes. Cabrero and Camacho (2008) investigated the regularities and restrictions in chromosome location of ribosomal RNA genes in grasshoppers by fluorescent in situ hybridization (FISH), and their phenotypic expression were assessed by nucleolus formation at first meiotic prophase cells, analyzed by silver impregnation, in 49 grasshopper species. High variation was found for rDNA location between species within most genera analyzed. The mean haploid number of rDNA loci detected by FISH was 2.47, but some species had up to 10 loci.

However, the chromosome distribution of rDNA loci differed between the Gomphocerinae and Oedipodinae subfamilies, most loci being proximal to the centromere in the former and distal to it in the latter. Chromosomes 2, 3 and X frequently carried rDNA in Gomphocerinae species with 2n
chromosomes, whereas chromosomes 6 and 9 were the most frequent rDNA locations in the Oedipodinae. About 13% of the 126 rDNA loci detected by FISH were silent. Comparison of FISH and silver-impregnation results suggested the existence of cryptic NORs. This was especially clear after the same cells in two species were sequentially treated with both silver impregnation and FISH. The abundance of silent and cryptic loci might thus suggest that rDNA spreads through grasshopper genomes by Dubcovsky and Dvorak mechanism. Based on the results it was concluded that the cryptic NORs might correspond to nascent NORs, whereas the inactive rDNA loci might correspond to those being in the process of elimination.

To investigate its controversial taxonomy and evolutionary history, Chintauan-Marquier et al., (2011) studied 19 species representative of its main tribes, and 7 Acridoidea outgroup species. More than 1650 base pairs of three regions of nuclear rDNA (18S, ITS1, 28S) and one mitochondrial rDNA (12S) were combined and used to construct parsimony, maximum likelihood and Bayesian phylogenies. Results correspond with the present geographical distribution of the taxa rather than the existing taxonomy based on morphological characters. The morphologically unclassified and atypical taxa group with the Neotropical Melanoplinae. Based on the results it appears that the currently recognized Melanoplinae are polyphyletic due to inclusion of the Mexican genus Netrosoma. Further, distribution of the American and Eurasiatic Melanoplinae fauna can be explained by climatic and geological
events, such as the Andean uplift, that would have affected the diversification and migration of Neotropical taxa.

**Restriction fragment analysis of mitochondrial DNA (mtDNA)**

Mitochondrial DNA is used for marker analyses largely because of their maternal inheritance, haploid status, and high rate of evolution. In insects, a further advantage of using mitochondrial markers is that many of these loci can be readily amplified by using universal primers designed from highly conserved mitochondrial genes. Chapco et al. (1994) used restriction fragment analysis of mitochondrial DNA (mtDNA) to examine genetic variation within and among 11 species of grasshoppers in the subfamily Melanoplinae. Total DNA of 236 individuals was digested with 10 restriction enzymes and probed with three cloned EcoRI fragments representing the entire mitochondrial genome of *Melanoplus sanguinipes*. The average size of mtDNA for these species was about 16.1 kilobases. Average nucleotide diversities within species ranged from 0.00 to 0.31 per cent.

Species regarded as major pests appear to have larger diversities. For each species, common genotypes occur throughout the sampled region suggesting very little spatial differentiation although in a couple of melanoplines, one or two haplotypes are geographically restricted. Comparisons among species led to a hypothesis concerning their phylogenetic relationships. Not all affinities accorded with expectations based on morphological comparisons. The estimated time of divergence for the genus Melanoplus is at least 4 million years.
Molecular phylogeny – a tool to resolve taxonomic disputes

The grasshopper subfamily Melanoplinae, comprising a large number of genera and species, maintains a distribution throughout Eurasia and the New World. Melanoplinae, comprising over 100 genera and 800 species (Otte 1995), represents a diverse assemblage of species that ranges throughout Eurasia and the New World (Vickery 1987, Otte 1995). Unfortunately, organization of taxa within the subfamily is unclear. Melanoploids have been divided into two (Vickery 1977), three (Rehn and Randell 1963), or seven tribes (Vickery 1997).

Recently, Perez-Gelabert and Otte (2000) have questioned the ability of morphological traits to resolve systematic disputes among the melanoplines; they go on to indicate that phylogenetic analyses of genetic data are required to resolve uncertainties. Despite various attempts to subdivide the subfamily, taxonomic assignments within the Melanoplinae remain unclear. However, the placement of Eurasian and related Nearctic genera within a separate tribe, Podismini, is a common feature within most proposed taxonomic schemes. The Podismini have been independently divided into two and four subtribes on the basis of cytogenetic and morphological traits, respectively; however, the two schemes differ with respect to the placement of certain genera.

Litzenberger and Chapco (2001) analyzed sequence data obtained from portions of four mitochondrial genes (cyt b, COII, ND2, and COI) to (1) test the hypothesis that Eurasian podismines are monophyletic and examine
which, if any, of the proposed subtribal affiliations is correct. The view that the nominate genus, *Podisma*, represents a form closely related to the ancestral podismine stock was also examined.

Using Parsimony, neighbor-joining, and likelihood tree construction methods Litzenberger and Chapco (2001) demonstrate strong support for claims of Eurasian podismine monophyly. Application of the molecular clock method places the separation from North American melanoplines at 62 mya. Neither cytogenetic nor morphological subtribal assignations of genera within Podismini is supported by molecular data and showed that *Podisma*, rather than occupying a basal position among the podismines as suggested in the literature, is a more recently evolved genus.

**Molecular Phylogeny a tool to establish phylogeographic divergence**

The grasshopper *Oedaleus decorus* is a thermophilic insect holds a large distribution range, spanning across Mediterranean regions in Europe to Central-Asia and China. In a study, Kindler et al. (2012) analyzed the extent of phylogenetic divergence and the recent evolutionary history of the species based on 274 specimens from 26 localities across the distribution range in Europe. Phylogenetic relationships were determined using sequences of two mitochondrial loci (ctr, *ND2*) using neighbor-joining and Bayesian methods. Additionally, genetic differentiation was analyzed using mt DNA and 11 microsatellite markers using F-statistics, model-free multivariate and model-based Bayesian clustering approaches.
Phylogenetic analyses detected consistently two highly divergent, allopatrically distributed lineages within *O. decorus*. Results depicted that the divergence among the Western and Eastern lineages meeting in the region of the Alps was similar to the divergence of each lineage to the sister species *O. asiaticus*. Genetic differentiation for ctr was extremely high between Western and Eastern grasshopper populations ($F_{ct} = 0.95$). Similarly, the microsatellite markers detected much lower but nevertheless very significant genetic structure among the selected population of insects used in the study.

Further, nuclear data also demonstrated a case of cytonuclear discordance because the affiliation with mitochondrial lineages was incongruent. Put together the results provided an evidence of an ancient separation within *Oedaleus* and either historical introgression of mtDNA among lineages and/or ongoing sex-specific gene flow in this grasshopper. Further, stressed the importance of multilocus approaches for unraveling the history and status of taxa of uncertain evolutionary divergence.

**Evolutionary history and taxonomy of a short-horned grasshopper (Melanoplinae)**

The Melanoplinae is one of the largest subfamilies of the Acrididae grasshoppers, with a Holarctic–Neotropical distribution. Chintauan-Marquier *et al.*, (2011) investigated its controversial taxonomy and evolutionary history using 19 species as representative of its main tribes, and 7 Acridoidea out-group species. More than 1650 base pairs of three regions of nuclear rDNA (18S, ITS1, 28S) and one mitochondrial rDNA (12S) were combined and used
to construct parsimony, maximum likelihood and Bayesian phylogenies. Results correspond with the present geographical distribution of the taxa rather than the existing taxonomy based on morphological characters. The morphologically unclassified and atypical taxa group with the Neotropical Melanoplinae. The currently recognized Melanoplinae appear to be polyphyletic due to inclusion of the Mexican genus Netrosoma. The distribution of the American and Eurasiatic Melanoplinae fauna can be explained by climatic and geological events, such as the Andean uplift, that would have affected the diversification and migration of Neotropical taxa.

**Molecular phylogenetic evidence for multiple dispersal**

The gomphocerine grasshoppers, comprising over 1000 species, occur on all continents excepting Australia. Contreras and Chapco (2001) using portions of four mitochondrial genes (coding for cytochrome b, cytochrome oxidase subunits I and II, and NADH dehydrogenase subunit V) phylogenetically analyzed using weighted and unweighted maximum parsimony, maximum likelihood and Bayesian methods. The study depicted that maximum resolution could be achieved using weighted parsimony (counting transversions at third codon positions only) and Bayesian methods, and treating all four sequences, totalling 1892 bp, as a unit.

Based on the results the subfamily is provisionally accepted as monophyletic. The tribe Chrysochraontini was found to be monophyletic, whereas the monophyletic status of Aulocarini and Dociostaurini remained unclear. Tribes, Arcypterini, Chorthippini and Gomphocerini, were not
monophyletic and hence warrant further scrutiny. Regarding biogeographic origins of the subfamily, the molecular data support Vickery's assertion that there were multiple periods of dispersal, most likely from Eurasia to North America. Assigning the range 50 to 70 Mya to the time of gomphocerine divergence correlates the times of these biogeographic events.

**Biogeographic analysis of mtDNA in grasshoppers**

The Melanoplinae constitute one of the two largest subfamilies of Acrididae. Distributed mainly throughout the New World and parts of Eurasia, this group of grasshoppers includes over 100 genera and 800 species. Over the past five decades there has been considerable speculation on the origins of North and South American taxa. The most favored hypothesis proposes an ancient division of Laurasian taxa accompanying the separation of North America and Eurasia, with subsequent radiations within those continents, followed by a recent incursion of Nearctic melanoplines into the southern hemisphere with the establishment of the Isthmus of Panama. Chapco (2001) tested the scenario by phylogenetic analysis using as characters portions of five mitochondrial gene sequences, totaling 2285 bp. Three tree-building methods, maximum-parsimony, neighbor-joining, and maximum-likelihood, results strongly support the different view that melanopline grasshoppers originated somewhere in the Americas and spread to the Old World.
Mitogenome of Orthoptera: Acrididae

The complete nucleotide sequence of the mitochondrial genome (mitogenome) of *Gomphocerus tibetanus* Uvarov, 1935 (Orthoptera: Acrididae: Gomphocerinae) was determined. It is 15,571 bp in length and contains 74.8% A + T. All *Gomphocerus tibetanus* protein-coding sequences start with a typical ATN codon. The usual termination codons (TAA and TAG) were found from 13 PCGs except COI and COII which took incomplete codon T as termination codons. All tRNA genes could be folded into the typical cloverleaf secondary structure, except tRNA\textsubscript{Ser} (AGN) lacking of dihydrouridine (D) arm. The sizes of the large and small ribosomal RNA genes are 1313 and 822 bp, respectively. The A + T content of the A + T-rich region is 82.3%.

Recently, Zhang *et al.*, (2013) elucidated the complete mitogenomes of *Asiotmethis zacharjini*, *Filchnerella helanshanensis* and *Pseudotmethis rubimarginis*. Data depicts that each of the grasshopper had 15,660 bp, 15,657 bp and 15,661 bp in size, respectively. All three mitogenomes contain a standard set of 13 protein-coding genes, 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs) and an A+T-rich region in the same order as those of the other analysed caeliferan species, including the rearrangement of trnAsp and trnLys. The putative initiation codon for the cox1 gene in the three species is CCG. The long polythymine stretch (T-stretch) in the A+T-rich region of the three species is not adjacent to the trnIle but inside the stem-loop sequence in the majority strand. The mitogenomes of *F. helanshanensis* and
P. rubimarginis have higher overall similarities. The characterization of the three mitogenomes will enrich our knowledge on the Pamphagidae mitogenome. The phylogenetic analyses indicated that within the Caelifera, Pyrgomorphoidea is a sister group to Acridoidea. The species from the Pamphagidae form a monophyletic group, as is the case for Acrididae. Furthermore, the two families cluster as sister groups, supporting the monophyly of Acridoidea. The relationships among eight acridid subfamilies were \((\text{Cyrtacanthacridinae} + (\text{Calliptaminae} + (\text{Catantopinae} + (\text{Oxyinae} + (\text{Melanopline} + (\text{Acridinae} + (\text{Oedipodinae} + \text{Gomphocerinae})))))))\).

**Application of DNA profiling in Ecosystem Management**

The diversity of insects in tropical forests remains poorly known, in particular regarding the critical feeding associations of herbivores that drive species richness. The methodological approach adopted for establishing feeding associations may affect the inferences drawn about host specificity and herbivore species richness. Diverse approaches have been used to obtain data on host associations and host preferences of insects, but all of them are time-consuming and have various limitations. Classical methods include observations of host use either *in situ* (Barone, 1998, 2000) or in laboratory tests (Novotny *et al.*, 2006; Dyer *et al.*, 2007), transplantation experiments (Eichhorn *et al.*, 2008) or behavioural tests by exposure to plant volatiles (Fernandez *et al.*, 2007).

Other studies have attempted the direct identification of the feeding source, either through morphological analysis of the gut content (Otte and
Joern, 1976), diet plant tissue-specific staining techniques (Schlein and Jacobson, 1999), or diet plant isotope analysis from gut contents (Post, 2002). Most analyses of insect host specificity in rainforests have been much less detailed. Early studies did not test feeding directly but used capture sites to establish host associations (Erwin, 1982). Studies based on direct feeding trials generally led to estimates of tighter host specificity than simple presence on a host plant (Lewinsohn and Roslin, 2008). This established the true nature of herbivore interactions, but the huge expense in manpower required (Weiblen et al., 2006) is prohibitive in most cases.

In addition, feeding studies of this kind usually concentrate on common herbivore species (Novotny et al., 2007), although most species in tropical forest assemblages are rare (Novotny and Basset, 2000), while artificial breeding conditions may alter insect behaviour and therefore result in inaccurate conclusions on host breadth. A further problem is that the taxonomic uncertainty in hyperdiverse insect groups due to numerous unnamed species which requires that specimens rather than names must be cross-checked among samples, which in turn adds great difficulties to analyses of herbivore data among independent studies (Lewinsohn et al., 2005). Finally, where species delimitation is incomplete and relies on preliminary morphospecies approaches, the finer details of host associations, including recently diverged races or species, may be missed altogether (Condon et al., 2008). DNA-based techniques can potentially solve the dual
problem of imprecise insect taxonomy and incomplete host plant data in a single step.

Specimens of folivorous leaf beetles have been shown to contain a ‘molecular record’ of their feeding source in the form of ingested plant material. Consequently, host plant DNA can be PCR amplified from a standard whole-body DNA extraction of herbivorous insects and identified against existing taxonomic DNA databases (Jurado-Rivera et al., 2009). This same DNA extraction is used to amplify diagnostic insect DNA fragments for a sequence based identification of the herbivore. A recent approach by Navarro et al (2010) pertaining to the analyses plant DNA ingested by herbivorous insects; direct PCR amplification from DNA extracts from weevils (Curculionoidea) using chloroplast (trnL intron) primers was successful in 41 of 115 cases, resulting in 40 different sequences. The technique provides a new means of studying species diversity and plant–herbivore interactions in tropical forests, and removes the constraints of the need for actual observations of feeding in ecological and evolutionary studies.

In addition, it had been proven by 18S rDNA (Liu and Jiang, 2005) and 16S rDNA (Liu et al, 2005; Sun et al, 2006) that Catantopidae, Arcypteridae, Gomphoceridae and Acrididae were non-monophyletic. At the molecular level, Liu and Jiang (2005) proposed that the above five families should be grouped into the family Acrididae in accordance with the international system. What are the phylogenetic relationships of some taxa within the Acrididae? Is each subfamily within the Acrididae a monophyletic
group? In this study, molecular phylogenetic trees will be reconstructed based upon the combined data of the 12S rDNA of selected species in Acrididae grasshoppers to reassess their phylogenetic relationships, so as to further clarify these unresolved issues and establish their intracontinental relationship based on evolutionary phylogenetics.