POST-EMERGENCE DEVELOPMENT
OF MALE GENITALIA
IN SCHISTOCERCA AMERICANA (DRURY)
(ORTHOPTERA: ACRIDIDAE: CYRTACANTHACRIDINAE)

A Thesis
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ABSTRACT

Post-emergence development of male genitalia in *Schistocerca americana* (Orthoptera: Acrididae: Cyrtacanthacridinae) was studied. The present study demonstrates for the first time that the internal skeletal structures in the male genitalia continue to develop after the adult emergence. Also, the taxonomic use of the genitalic characters is reevaluated.

An experiment was set up to examine the developmental patterns in the male genitalia and female ovipositor. The mechanism of the genitalic apodeme development is explained by the resilin deposition possibly affected by bursicon secretion. Overall, the structures affected by the cuticle deposition are the apodemes providing muscle attachment sites that enable the necessary movement during copulation. Non-changing structures in the genitalia may be directly involved in the internal courtship during copulation. Therefore, sexually immature individuals can be considered functionally incapable of copulation because the necessary structures have not been fully matured. In order to test if the post-emergence development is widespread, the different aged specimens of *Schistocerca gregaria* (Forskål) and *Locusta migratoria* (Linnaeus) were studied. Although the morphology was different between *Schistocerca* and *Locusta*, the overall developmental patterns were similar.
The sexual maturation period of *Schistocerca* takes at least 30 days after the emergence. The life history theory predicts that the age at maturity is shaped by the trade-off between survival and reproduction. In *Schistocerca*, the structures associated with the flight mature earlier in adult development whereas the genitalia continue to develop for about thirty days after emergence. Delayed maturation is well explained by the life history theory when considering that the flight may be the most important trait for survival and that the reproduction is usually costly.

A morphological phylogeny of the North American *Schistocerca* is proposed for the first time. The monophyly of the *alutacea* complex *sensu* Dirsh is not supported. I argue that the previous subspecies of *S. alutacea* are morphologically distinct species. The origin of *S. gregaria* and the evolution of swarming behavior are briefly discussed in the light of the present phylogenetic hypothesis.
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Some of the ideas in the thesis came from lengthy, but friendly discussions with my fellow graduate students, especially, Marc Branham, Eric Dotseth, and Kurt Pickett.

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B: The New World origin hypothesis. The common ancestor of *Halmenus* and *Schistocerca* colonized the New World once, and *S. gregaria* re-colonized the Old World.
Although the final molt is often interpreted as the terminal event in insect development, the adult stage is in fact an active period of development in the life of an insect. Crucial aspects of reproduction may be determined after the last ecdysis. In this chapter, I review three separate topics relating to the development of male genitalia in Schistocerca. First, I discuss the physiological changes in the post-emergence development period, including cuticular, muscular, ultrastructural, and hormonal changes. In the second section, the biology of Schistocerca is reviewed. Life history theory is discussed in terms of the trade-off between survival and reproduction. Last, I discuss the taxonomic use of genitalia and its historical basis.
1.1. POST-EMERGENCE DEVELOPMENT IN *SCHISTOCERCA*

There is a conventional belief that the adult instar is immutable because all the necessary changes occur with the final molt. However, this is far from true. Many insects experience a post-emergence developmental period, commonly called a teneral period, during which they experience significant physiological and biochemical changes. The sexual maturation period in *Schistocerca* Stål depends somewhat on temperature and light, but in general it takes about 2-3 weeks (Weis-Fogh, 1952). Here I summarize developmental patterns at various levels. Most of the previous work focused on the infamous desert locust, *Schistocerca gregaria* (Forskål), but given the close phylogenetic relationship with *S. americana* (Drury) (Harvey, 1981), they are relevant to present study.

1.1.1. Cuticular development

Cuticle continues to be deposited and develop internally after the final molt. Cuticle and flight muscles of newly emerged locusts contain only one-third of the dry matter found in fully developed individuals (Weis-Fogh, 1952). This suggests that there is a significant growth during the teneral period. Neville (1963a) described adult cuticular growth in *Schistocerca*. He suggested that the tibia is thickened toward the inside by the secretion of successive layers of cuticle from the epidermis. He showed that layers of cuticle are deposited daily and the exact age of an insect after the final molt could be determined by counting the number of layers in the cross section of a leg. This pattern is equivalent to helicoidal chitin layers deposited at night and unidirectional
chitin layers deposited during the day (Neville, 1993). This phenomenon is widespread in Insecta, present in Orthoptera, Blattaria, Phasmida, Dermaptera, Odonata, Hemiptera, Homoptera, and Hymenoptera (Neville, 1983). Neville (1963b, c) showed that the daily cuticular growth is also found in the thoracic apodemes of S. gregaria, which have resilin-based cuticle (chitin crystalites set in a matrix of the protein resilin). Schlein (1972) also showed that the male genitalia of the fly Sarcophaga falcultata Pandelle are resilin-based and undergo cuticular development after the final molt. Resilin has a highly elastic property, and thus is ideal for muscle attachment sites that experience contraction constantly (Neville, 1993). However, the growth of resilin during maturation is different from that of exoskeleton. Chitin deposition in resilin cuticle is quantal in nature, which means that a constant weight of chitin is secreted daily by a constant population of epidermal cells (Neville, 1963c). Chitin deposition ceases in the thoracic apodemes one week after emergence (Neville, 1963c). Compared to endocuticle deposition in exoskeleton which lasts until sexual maturity, the resilin deposition in thoracic apodemes is remarkably short in duration.

Bursicon is a neurosecretory hormone released by the ganglia of the central nervous system that controls tanning and the mechanical properties of insect cuticle during and after molting (Fogal and Fraenkel, 1969). Fraenkel and Hsiao (1965) demonstrated that juvenile hormone and ecdysone do not play a role in tanning, and bursicon is responsible for sclerotization. Fogal and Fraenkel (1969) showed that bursicon induces melanization of the outer layers of the cuticle as well as deposition of post-emergent endocuticle in a flesh fly, Sarcophaga bullata (Parker). Given that all internal apodemes are composed of resilin cuticle (Neville, 1993), it is likely that
bursicon (or similar neurosecretory hormone) is responsible for *Schistocerca* apodeme development. Because bursicon travels through haemolymph (Fogal and Fraenkel, 1969), one would expect that the cuticle development rate should be similar throughout the insect body. However, endocuticles in exoskeleton and thoracic apodemes of *S. gregaria* clearly have different maturation time (Neville, 1963a, c). This different behavior can be achieved if the epidermis itself can discriminate and respond to bursicon independently.

1.1.2. Muscular development

Important muscular development also takes place after the final molt (Weis-Fogh, 1952). Indeed muscle development in the adult instar is known in several orders of insects, and is especially well-documented in Diptera due to its medical importance. Bursell (1961) showed that thoracic flight muscles of the tsetse fly (*Glossina swynnertoni* Austen) complete development after the second or third blood meal. A cross-section of the thorax showed a significant difference in muscle mass between freshly emerged flies and fully mature flies. The muscle development is accompanied by an increase in the volume of mitochondria as well as contractile protein (Finlayson, 1975). In *Calliphora erythrocephala* Meigen, the number of thick filaments in a single myofibril of a dorsal longitudinal fiber increases from 669 to 1186 at emergence to a maximum of 2000 after 10 days at 25 °C (Finlayson, 1975). *Schistocerca americana* appears to exhibit a similar developmental pattern. A freshly molted adult has a soft thorax and is unable to fly, but the older adult has a very firm thorax and is a strong flyer. This seems to correlate well with the thoracic apodeme development because as muscle
fibers develop the corresponding apodemes also need to increase in size to provide attachment sites (Schlein, 1972).

1.1.3. Ultrastructural development

Post-emergence development is not limited to apodemes and muscles, but also found at the level of ultrastructure. Odhiambo (1966a) showed that the volume and hormonal activity of the corpora allata of the adult *Schistocerca gregaria* males progressively increases during sexual maturation. The volume change is achieved by the development of nuclei and greater content of allatum-cell cytoplasm (Odhiambo, 1966b).

Membranous structures in male genitalia also develop during maturation. Viscuso et al. (1985) showed that the epithelium of the anterior region of the ejaculatory duct in a grasshopper, *Eyprepocnemis plorans* (Charpentier) (Acrididae), undergoes significant cellular differentiation. The changes are the result of the release of Golgi vesicles and of lysis of cells. The cellular development was only found during sexual maturation of the adult, and not in the newly emerged or sexually mature adults (Viscuso et al., 1985).

Gonads develop during maturation as well (Norris, 1954). Norris (1954) found that the yolk deposition in the eggs and the growth of the egg-rudiments were still in the process in 3-week-old female *S. gregaria*. He also showed that the receptaculum seminis of the newly emerged males contained no spermatozoa and the accessory glands contained no secretion. Production of spermatozoa and accessory gland secretion begins as early as five days after emergence (Norris, 1954). Therefore, it is clear that the
necessary gametes for the fertilization are not fully mature at emergence and continue to develop throughout the adult maturation.

1.1.4. Role of endocrine system during development

Juvenile hormone (JH) is the most versatile hormone in insect development, affecting metamorphosis, reproduction, diapause regulation, phase polymorphism, and more. Locusts have density-dependent phase polymorphism in which solitary nymphs are green and gregarious ones are black and red. In swarming locusts, JH is known to induce a green color in solitary nymphs and to affect the production of maturation-accelerating pheromone to a lesser extent (Pener, 1998).

In an adult stage however, JH only plays an indirect role. In the gregarious phase, locusts turn from brown to bright yellow as they mature, whereas solitary ones do not. Loher (1959) found that a mature, yellow male is able to accelerate the maturation process of immature males by secreting a volatile substance. The source of this volatile chemical are the corpora allata (Loher, 1960). Allectomized males were unable to turn yellow and to mate. Implantation of corpora allata into allectomized males induced both yellowing and sexual maturation. This finding is congruent with Odhiambo's (1966b) finding of volume increase in corpora allata discussed above. Mahamat et al. (1993) demonstrated that the volatile chemical from mature males is phenylacetonitrile. Production of both the maturation-accelerating pheromone and the sexual maturation itself is under the control of corpora allata and the JH (Pener, 1998), but the exact effect of JH on production of phenylacetonitrile has not been demonstrated yet.
1.1.5. Summary

Overwhelming evidence suggests that the adult instar is a developmentally active period and cuticle, muscles, and cells experience structural changes. Table 1.1 summarizes the post-emergence development at different levels.

Metamorphosis allows insects to undergo a major morphological reconfiguration, but in many cases adult instars are far from functionally mature. In simple terms, grasshoppers cannot fly just because they have wings. Corresponding changes in flight muscles, thoracic apodemes and sclerotization of external cuticle are necessary to make flight possible. Similar reasoning applies to the genitalia. Because the copulation involves extruding the phallic complex and pumping spermatophore through the endophallus, locusts need to have fully functional muscles and flexible genital apodemes to copulate. In terms of sexual maturation, membranous structures such as the corpora allata and ejaculatory ducts need to be mature in order to secret necessary hormones (Viscuso et al., 1985), and the gonads need to fully develop in order for fertilization to occur (Norris, 1954).
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Table 1.1: Post-emergence development of different structures and their processes and timing along with orders known to have the phenomena.
1.2. EVOLUTION OF LIFE HISTORIES IN *SCHISTOCERCA*

*Schistocerca* species mature late in the adult life, usually about 30 days after emergence, which is an unusually long period comparing to other insects (Norris, 1954; Uvarov, 1966). What determines the age at maturity? Besides the physiological and developmental explanations described in the previous section, an evolutionary explanation for the delayed maturation in *Schistocerca* deserves attention. In life history theory, an optimal age at maturity is attained where the benefits and costs of maturation at different ages balance at a stable equilibrium point (Stearns, 1992). In this section, I describe the life history of *Schistocerca* compiled from literature, and suggest factors that select for the delayed sexual maturation under the framework of the life history theory.

1.2.1. Biology of *Schistocerca*

The genus *Schistocerca* is famous for swarming locusts recorded in the Bible and Koran (Dirsh, 1974). Although there are four agriculturally important locusts in the genus, the rest are sedentary grasshoppers. North American sedentary *Schistocerca*, in particular, never swarms despite their strong flight capacity. Some *Schistocerca* have one generation per year and others have two (Capinera, 1993). Some overwinter as adults and others do so as eggs (Uvarov, 1966). Some are arboreal and others are desert-dwelling (Dirsh, 1974). Some are generalists and others are specialists. At least one species, *S. ceratiola* Hubbell and Walker, is nocturnal, whereas others are diurnal.
(Hubbell, 1928). Despite these differences, Schistocerca species share a similar developmental pattern and mating biology.

Schistocerca usually has five to six nymphal stages (Uvarov, 1977). Schistocerca gregaria and S. americana nymphs have different colors depending on the population density. Solitary nymphs are usually green, and gregarious ones are black and red. This density-dependent pattern is also observed in sedentary species such as S. lineata Scudder. Sword et al. (2000) has shown that the nymphs with aposematic coloration preferentially feed on the toxic plants, and as a result, predators associate the unpalatability with the aposematic coloration. However, this aposematic pattern has not been studied in other sedentary species.

Adults of North American species can be seen in early autumn (Squitier and Capinera, 2002), and species from Central and South America can be found almost all year round. After the adult emergence, locusts go through an intense feeding period (Norris, 1954). In swarming species, immature adults mainly feed and migrate, and mature ones mainly mate and reproduce (Uvarov, 1977). Schistocerca gregaria and S. americana can begin to fly two days after emergence, but full flight capacity is reached one week after final emergence (Weis-Fogh, 1952). Sexual maturation takes from three to five weeks, depending on temperature (Loher, 1960). Swarming species turn bright yellow at sexual maturity in high density (Norris, 1952, 1954).

Mating behavior in Schistocerca is different from other grasshopper species with sexual communication. Oedipodine grasshoppers (band-winged grasshoppers) have a sexual display flight, and acridine grasshoppers (slant-faced grasshoppers) have acoustic communication. Schistocerca lacks any sexual communication (Otte, 1970). Loher
and Otte (1970) studied the mating biology of *Schistocerca* in detail. Mature males stealthily approach and jump on the back of females. Females resist by pushing males with the hind legs or by shaking the body. Males that hold on to females bend down their abdomen to copulate, and once copulation is initiated, the pair stays together from 10 minutes to a few hours. Copulation only occurs between mature individuals, but when a mature male attempts to copulate with an immature female, the female violently shakes off the male (Norris, 1954). During copulation, males produce a disturbance sound by rubbing the forewing against the hindwing when other males approach the copulating pair (Loher, 1960). Females tend to mature faster than males (Norris, 1954), and both males and females mate multiple times (Loher, 1960; Otte, 1970). In *S. americana*, females tend to oviposit usually within one day after the copulation (personal observation), but Norris (1954) noted that the average interval between copulation and oviposition in *S. gregaria* is about six days. The average longevity of adult *Schistocerca* depends on the environment, but individuals continue to live about two months after the copulation and oviposition (Norris, 1954). Several tropical species can live up to eight months as adults (Uvarov, 1966).

1.2.2. Evolution of age at maturity

Many hemimetabolous insects have a long sexual maturation period (Ridley, 1989). Almost all grasshopper species take a considerable time between emergence and reproduction, and this has been explained by the duration of gonad development (Uvarov, 1977), but cuticle and muscle also develop during sexual maturation (Neville, 1963a). Comparing to the holometabolous insects, the sexual maturation in *Schistocerca*
is very long. What is the possible adaptive reason for the long maturation period? Theory of life history offers several explanations in terms of trade-off between two or more life history traits involved in maturation (Charlesworth, 1994; Roff, 1992; Stearns, 1992).

Age at maturity is defined as age at first reproduction (Stearns, 1992). Selection favors reproducing early in life when there is high adult mortality because the offspring of the early maturing organisms are born earlier and start reproducing sooner (Roff, 1992). For example, fruit flies (Drosophila melanogaster) living under high mortality regime matured earlier and had shorter life spans (Stearns et al., 2000), and Cichoń (2001) explained this as a result of optimal resource allocation during adult life. However, if there is a higher cost in reproduction than in survival, the age at maturity is delayed (Stearns, 1992; Zera and Harshman, 2001). Sgro and Partridge (1999) selected for fruit flies with very early oviposition (young line) and very late oviposition (old line), and showed that there was a higher mortality in the young line as a result of damages from the early reproduction. If there is a limited common resource pool to maintain the costs for two life history traits, then a trade-off should result (Zera and Harshman, 2001).

All Schistocerca species are strong fliers, and it is likely that there is a low adult mortality rate from the predation. Adult instars are polyphagous (Sword and Dopman, 1999), which suggests that they do not have particular chemical protection. Therefore, the flight is perhaps the most important trait associated with the survival. Flight enables grasshoppers to quickly escape from predators and to locate food source faster (Farrow, 1990). Especially in locusts, achieving the full flight capacity earlier in adult life is advantageous for the swarm dynamics (Steedman, 1990). In S. gregaria, the flight
muscle and apodemes complete development within one week after emergence (Weis-Fogh, 1952; Neville, 1963a) whereas the sexual maturation continues for three more weeks (Norris, 1954). If there is a common resource pool for developing flight-related structures and reproduction-related structures, the locust should allocate the resource in order to maximize its life history strategy. Indeed, both flight muscle synthesis and gonad development are directly related to the nutrient uptakes (Norris, 1954). Therefore, the delayed maturity in Schistocerca can be achieved as a result of trade-off between two important life history traits, survival and reproduction.

What are the possible benefits of delayed maturity in Schistocerca? The age at maturity can be delayed if it permits the organism to grow larger, to live longer, to have higher fecundity later in life, or to gain in lifetime reproductive success (Stearns, 1992). Although insects do not increase in size during adult life, internal growth of several anatomical features such as flight muscle and internal skeleton occurs (Weis-Fogh, 1952; Norris, 1954). Delayed maturity permits the insects to utilize internal resources for the development of essential structures for mobility, which in turn enhances the chance of survival. Sexually immature individuals are fully mobile because the flight muscles complete development within one week after emergence (Weis-Fogh, 1952; Neville, 1963a). The reason for the delayed development of the flight-related structures is not clear, but the delay could simply be the intrinsic property of hemimetabolous insects. During the sexual maturation, locusts spend most of the time feeding to increase the fat content and to nourish their gonads (Norris, 1954; Uvarov, 1966). Thus, delayed maturity in essence prepares well-nourished gametes which ensures higher fecundity later in life (Stearns, 1992).
Delayed maturity may also be beneficial in terms of the mating behavior. Although pheromones are involved, *Schistocerca* mating is mostly the result of visual recognition (Loher, 1959). In the gregarious phase, the group mating has been observed frequently (Steedman, 1990), which indicates that finding a mate is relatively easy. However, solitary or sedentary *Schistocerca* individuals are scattered around, and they may have to actively search for the mate. Therefore, the primary challenge for *Schistocerca* is for females to survive long enough to gain mass and for males to survive long enough to mate many such females.

In order for a trade-off to occur, the benefit of survival has to balance with the cost of reproduction (Zera and Harshman, 2001). During sexual maturation, both male and female concentrate on feeding (Steedman, 1990), but females tend to mature earlier than males (Norris, 1952). Stearns (1992) explained that this bimaturism could be found in organisms in which males do not control access to females and the early maturation of females is favored because females gain fecundity with size at a higher rate than males. The total body weight of females drastically increases before oviposition because of the fat body increase and the ovary development (Norris, 1954) and this possibly means less mobility and more vulnerability. During oviposition, females stretch their abdomens up to fifteen times their original length (Vincent, 1981). Although the abdominal membranes recover (Neville, 1993), this is a time-consuming event that exposes females to the danger of predation. Therefore, it is advantageous for females to delay their sexual maturation to avoid the high cost of reproduction. As a result, it is likely that the benefit of survival outweighs the benefit of reproduction, and it is more advantageous for *Schistocerca* to delay reproduction.
Does delayed maturation happen in other insect groups? Ridley (1989) compiled the mating frequencies and timing of mating in different insects. Unfortunately, he did not specify the exact time of mating, and only made two categories: immediately after emergence and after a period of maturation. Although there are many exceptions, holometabolous insects, especially Coleoptera, Hymenoptera and Lepidoptera, tend to mate soon after emergence. Sexual maturation is extremely brief or may even be absent because all the necessary development occurs in a pupal stage or earlier. For example, male beetles are commonly observed to mate with freshly emerged females that have not yet completed tanning (Ridley, 1989). On the other hand, all orthopteroid insects mate after a period of maturation. Therefore, the adult teneral period in hemimetabolous insects may be functionally analogous to pupal stage in holometabolous insects in terms of sexual maturation. Not all hemimetabolous insects have long maturation period, however. Habrochila laeta (Hemiptera: Tingidae) mates immediately after emergence (Ridley, 1989), which shows that maturation period can be brief or non-existent.

1.2.3. Summary

Life history theories suggest that the age at maturity can be shaped by the trade-off between survival and reproduction. In grasshoppers, if flight is an important factor for survival, and ensures later success in reproduction, delayed maturation can be beneficial. High cost of reproduction in grasshoppers permits the delayed maturation as well.
1.3. TAXONOMIC USE OF GENITALIC CHARACTERS

In insect taxonomy, male genitalic characters are extremely useful for species identification. A random survey of taxonomic studies in two volumes (V. 93 (2000) and V. 94 (2001) and 31 taxonomic studies) of *Annals of the Entomological Society of America* reveals that most taxonomic descriptions across all insect orders (28 studies) include figures of male internal genitalia (at least one figure of aedeagus from Diptera, Hemiptera, Lepidoptera, Neuroptera, Coleoptera, Strepsiptera, and Plecoptera). These taxonomic studies found many useful characters from the male genitalia when describing a new species. In Acridoidea, many species have a distinct male genitalic morphology that can be uniquely identified (Hubbell, 1932, 1960; Dirsh, 1956, 1973; Cohn and Cantrall, 1974). In the sibling species of *Schistocerca alutacea* (Harris), male genitalia are the only reliable characters for species determination (Hubbell, 1960). Roth (1970) also suggested that externally similar species of cockroaches often have unique species-specific genitalia. Among orthopteroid insects, in particular, female internal genitalic characters are often considered taxonomically less reliable than male genitalia (Kevan, Akbar, and Chang, 1969) though exceptions exist (De Assis-Pujol and Lecoq, 2000). Lockwood (1989) criticized the use of male genitalia in acridid taxonomy due to the fact that they only apply to males. While this is obviously true, in many cases there is simply a limit to the convenience of ambisexual characters (Cohn, 1994). Male genitalia are not a panacea to all taxonomic problems. Modern taxonomists study morphological, biological, ecological, phenological, physiological, and molecular characters to delimit a species, but the utility of male genitalia in taxonomy is undoubtedly great.
1.3.1. History of internal genitalia use in Orthoptera

Early orthopterists did not use internal skeletal male genitalia as a taxonomic character because of several reasons (Hubbell, 1932). First, there were plenty of good external characters to differentiate species. They used colors and morphometrics, and these characters were enough to describe a species (for example, see Scudder (1899) for the revision of *Schistocerca*). Second, taxonomists were reluctant that they might damage the specimens by dissection. Species were often described based on single specimens, and taxonomists tried their best to minimize damage in type material. Third, many species were described based on female specimens, which obviously lack male genitalia. However, since the utility of male genitalia was realized, nearly all taxonomic studies of grasshoppers now describe the male genitalic morphology (Hubbell, 1932; Dirsh, 1956, 1974; Cohn and Cantrall, 1974).

Although not used taxonomically, the skeletal genitalic structures were the subject of many morphological studies in the early 20th century in order to establish the homologies of genitalic structures across the insect orders and to determine the origin of male genitalia (Crampton, 1918; Snodgrass, 1931, 1933; Michener, 1945). Else (1934) followed the life history of *Melanoplus differentialis* (Thomas) to test the idea that the male genitalia have originated from embryonic abdominal appendages, and suggested that the copulatory organs originated from the tenth rudimentary abdominal appendages. Quadri (1940) also suggested that the paired penis valves represented the appendicular outgrowths of the tenth abdominal segment. Matsuda (1958), however, argued that male genitalia in holometabolous insects could not be traced to rudimentary appendages and proposed that the origin of male genitalia is independent in different taxa. Scudder
(1971) listed abdominal appendage origin, sternal origin, and independent origin of male genitalia, and concluded that the male genitalia could have a mixed origin. It should be noted that the terminology used by morphologists suggested supposed homologies, but taxonomists used more descriptive terminology specific for the taxa they were studying (Scudder, 1971).

Hubbell (1932) was one of the first orthopterists to use male genitalia in a taxonomic study. North American genus *Melanoplus* Stål contains more than 200 species, and the species groups are easy to differentiate based on external morphology. However, Hubbell noticed that within close species groups, external morphological characters were often highly variable, and the internal male genitalia were the only reliable character for the species delimitation. Since Hubbell’s (1932) landmark study, taxonomic studies of grasshoppers began to include descriptions of male genitalia. Dirsh (1956) described the phallic complex of 778 genera of Acridoidea, and found the taxonomic utility of male genitalia in higher classification. Although male genitalia are highly species-specific in many genera, Dirsh (1973) expressed the view that the phallic complex of closely related species can be very similar in some genera with a recent origin (*Acrida* Linnaeus, *Truxalis* Linnaeus, and *Schistocerca*). However, Hubbell (1960), in his detailed study of North American *Schistocerca*, showed that there were still good taxonomic characters in male genitalia of closely related species.

Several attempts have been made to understand the higher relationships within Acridoidea based on the phallic complex. Dirsh (1956) grouped eight families in Acridoidea, but the relationships among families were poorly understood. Amedegnato (1976) concluded that the suborder Acridomorpha was characterized by a three-layered
phallic complex (epiphallus, ectophallus, and endophallus) and Acridoidea was the most advanced group because each layer is highly elaborated. Most recently, Eades (2000) suggested more resolved relationships among families based on presumed synapomorphic changes. However, none of the phylogenies was derived from a cladistic analysis, and more in depth studies are necessary.

1.3.2. Variability of male genitalia

When using male genitalia in a taxonomic or phylogenetic study, researchers usually make one important yet dangerous assumption, that is, male genitalia are invariable and stable within a species (Eberhard, 1985). This assumption needs to be tested, however, because male genitalia are still morphological characters which require a study of large samples.

Linsley (1939) was one of the first taxonomists to address potential variations in male genitalia. He studied the genitalia of the common checkered skipper, *Pyrgus communis* Grote (Lepidoptera: Hesperiidae) and found that genitalia do have a certain amount of variability. Yet, these variations were merely of size, not drastic morphological differences. Shapiro (1978) warned against the blind use of male genitalia in taxonomy citing Müller’s (1957, in Shapiro, 1978) experiments with the leafhopper male genitalia. Müller found that the morphology of male genitalia of leafhopper genus *Euscelis* (Cicadellidae) is affected by the daylength. As a result, two species described by the genitalic morphologies were considered to be one species with high phenotypic plasticity. Johnson (1995) discovered that there is a bimodal distribution of male styli length in the populations of mecopteran *Merope tuber* Newman.
Although the reason for variation is not known, he suggested food availability, allometry, and mating biology as potential reasons. Eberhard (1985) suggested that a taxonomically useful structure only needs be relatively invariable intraspecifically. However, seemingly invariable structures such as male genitalia do vary, and therefore it is important for taxonomists to use these characters with caution.

There are practical issues concerning the use of male genitalia as well. Unlike other external characters, internal male genitalia often require dissection and removal of muscle tissues for characterization (Hubbell, 1932; Dirsh, 1956). While certain parts of the male genitalia, such as cingulum, can be easily studied by extrusion of male genitalia using a probe (Cohn and Cantrall, 1974), in order to characterize the intromittent organ such as the endophallus, one needs to completely remove the structure from the insect. Because this procedure is destructive, it is conceivable that the taxonomists would be reluctant to dissect many specimens, especially when the materials are borrowed. In fact, the survey of taxonomic studies in two volumes of the *Annals of the Entomological Society of America* described above revealed that the figures of the male genitalia are either drawn from a single specimen or drawn to accommodate the intra-specific variations.

Taxonomic studies concerning the genitalic variations generally lack a developmental aspect. The only developmental studies concerning male genitalia dealt with embryonic limbs to hypothesize the origin of the male genitalia (Else, 1934). Because there have been no developmental studies concerning the skeletal changes in the male genitalia, it is likely that taxonomists were not aware of the developmental variations. However, as described in previous section, the adult stage may be a very
active stage of development which involve cuticular and muscular growth (Weis-Fogh, 1952; Norris, 1954; Neville, 1983). If the genitalic morphologies of different adult ages are significantly different, a taxonomist without the knowledge of the post-emergence development would be misled when characterizing the male genitalia. In hemimetabolous insects in which the adult cuticular development is likely (Neville, 1963a; Ridley, 1989), it is thus important to consider the developmental aspect before using the genitalia as a taxonomic character.

1.3.3. Male genitalia vs. female genitalia

Female genitalia have been used in taxonomic studies though less frequently than male genitalia (Kevan, Akbar, and Chang, 1969; Eberhard, 1985; Shapiro and Porter, 1989). In Acrididae, the external female genitalia such as the ovipositor are not sufficiently variable among species to be taxonomically useful (Dirsh, 1974). Internal structures, such as the bursa copulatrix and spermatheca, often contain important characters, but they are less commonly used because these are soft membranous structures that require a more careful preparation comparing to the male genitalia (Silfer, 1939; Cohn and Cantrall, 1974; Naumann, 1988).

The utility of female genitalia certainly depends on taxa. Fergusson (1988) studied the ovipositors of the several families of Cynipoidea (Hymenoptera) and concluded that the modification of the ninth tergite is group-specific and has a phylogenetic importance. Slifer (1939, 1940a, 1940b, 1943) conducted a series of morphological studies on the female genitalia of all grasshopper subfamilies. She revealed that each subfamily has a characteristic ovipositor, and genus-specific internal
structures. Naumann (1988) studied the internal female genitalia of fifteen species of Zygaenidae (Lepidoptera) and found that there are species-specific differences in the morphology of pseudobursa and Peterson's glands. Recently, De Assis-Pujol and Lecoq (2000) showed the species-specific differences of spermathecae in the gomphocerine grasshopper, *Rhammatocerus* Saussure.

In some cases, the morphology of the female genitalia directly corresponds to that of the male genitalia. Cohn and Cantrall (1974) showed that the length of the bursa copulatrix corresponds to the length of the aedeagus. Sota and Kubota (1998) studied the heterospecific mating of two closely related species of a ground beetle (Carabidae) that share a narrow hybrid zone, and demonstrated that male genitalia of one species caused the rupture of the vaginal membranes in the female of another species. These studies indicate that at least in some insects, the female genitalia mechanically correspond to the male genitalia. However, in many cases, there is no strong correlation between two sexes, and female genitalia are quite uniform among species (Eberhard, 1985; Shapiro and Porter, 1989).

1.3.4. Evolution of male genitalia

What we observe in nature is the enormous diversity of male genitalia. Eberhard (1985) listed several hypotheses that attempt to explain this phenomenon. The oldest one is the lock-and-key hypothesis which attempts to explain the species-specific genitalia in terms of mechanical reproductive isolation (Shapiro and Porter, 1989). However, this hypothesis fails to account for the invariable female genitalia found in numerous cases where the male genitalia are species-specific (Eberhard, 1985). Also, due to the lack of
empirical evidence, the lock-and-key hypothesis is largely disregarded (Eberhard, 1985) although there are rare cases where it is supported (Sota and Kubota, 1998). The genitalic recognition hypothesis states that the male genitalia are designed to stimulate the female, but Eberhard (1985) dismissed this hypothesis because the premises are similar to the lock-and-key hypothesis. Pleiotropism is another hypothesis which is largely untested. Mayr (1969) proposed that the genitalia are pleiotropically affected by many genes and shaped by the changes in the genetic constitution. The weakness of this hypothesis arises from the fact that the pleiotropism affects other internal organs, not just genitalic characters (Eberhard, 1985). The mechanical conflict-of-interest hypothesis is different from other hypotheses in that it deals with the reproductive interest of one sex over another. Arnqvist and Rowe (2002) recently tested this idea using the sexually antagonistic interactions of water striders (Hemiptera: Gerridae). They demonstrated that the evolutionary changes in species-specific genitalic morphologies are the consequences of the antagonistic sexual coevolution. Finally, Eberhard (1985) proposed the sexual selection by female choice as a plausible hypothesis for the diversity of male genitalia. He suggested that one of the functions of male genitalia is the internal courtship, and the diversity of male genitalia is the result of runaway selection generated by the female choice (Eberhard, 1985, 1993).
1.3.5. Summary

Male genitalia have been used extensively in taxonomy because of their enormous variability and often species-specific characters. Taxonomists should be aware of the variability of male genitalia, because the use of genitalic characters without the knowledge of environmental or developmental effect on the genitalic morphology can be misleading. The use of female genitalia depends much on the taxa, but they are generally less used by taxonomists because of practical reasons. Evolution of male genitalia is still a hotly debated subject, and the sexual selection by female choice hypothesis is among the most plausible hypotheses.
Male genitalia of Acrididae (Orthoptera) are divided into two main parts: external and internal genitalia. External genitalia refer to the terminal abdominal structures visible externally such as supra-anal plate (epiproct) and subgenital plate (9th abdominal sternum). These are modified tergum and sternum, and their primary function is to protect internal genitalia. The overall morphologies of external genitalia are similar among Schistocerca species (Dirsh, 1974). Internal genitalia, commonly called the phallic complex, are involved in copulation and spermatophore transfer, and they are situated in the last abdominal segment. The phallic complex of the grasshoppers is a combination of the skeletal and membranous parts, and the whole organ is derived from the ectoderm (Dirsh, 1956; Kevan, Akbar, and Chang, 1969). There are three distinct skeletal parts in the grasshopper phallus: endophallus, ectophallus, and epiphallus. Endophallus is the heavily sclerotized structure consisting of a pair of valves and the endophallic sac. Ectophallus encapsulates endophallus, and consists of cingulum and
ectophallic sclerties. Finally, epiphallus is a strongly sclerotized sclerite, located on the dorsal part of the phallic organ and below the supra-anal plate. Each components of the phallic complex is connected by membranes. I follow Dirsh’s (1974) terminology and the specific morphology and terminology of Schistocerca phallic complex are described below.

2.1.1. Endophallus

The skeletal part of the endophallus (Fig. 2.1) is a highly complex structure. Apical valve of penis (Ap), which penetrates the female counter-part, is distally located and slender. It is obtusely curved upward. Above the apical valve of penis is arch of cingulum (Ac), a globular sclerite which holds endophallus and cingulum together. Arch of cingulum is composed of two parts: distal projection (bulbous sclerite) and the base that narrows down to the valve of cingulum (Cv). Apical valve of penis is connected to the flexure (Fx) by a slender and curved “neck.” Proximal portion of endophallus is a wing-shaped basal valve of penis (Bp). Gonopore processes (Gpr) are a pair of ventral processes extending from the basal valves of the penis, and ejaculatory sac (Ejs) is attached ventrally. Ejaculatory sac is a membranous structure composed of ejaculatory duct and spermatophore sac. All the grooves are muscle attachment sites to enable the pumping motion to aid the spermatophore transfer.

2.1.2. Ectophallus

The most conspicuous structure is the cingulum, a strongly sclerotized organ that houses the endophallus (Fig. 2.2A). Dorsal portion of cingulum is the U-shaped
apodemes (Apd). Distal part of cingulum, where it is connected with endophallus through the arch of cingulum, is a round cover, which consists of zygoma and rami. Zygoma (Zyg) is a transversed dorsal part of cingulum, internally connecting to the arch of cingulum. Rami (Rm) is the lateral part, weakly sclerotized and often bulbous. The valve of cingulum (Cv) and the apical valve of penis are exposed through the ventral portion of zygoma.

Ectophallic sclerites (Fig. 2.2B) are a separate structure connected to the cingulum by membrane. It is a U-shaped structure which is situated ventrally to the cingulum. The probable function is to protect the apical valve of penis.

2.1.3. Epiphallus

Epiphallus (Fig. 2.3) is located dorsally to the cingulum. It is composed of wide bridge (B) and two triangular anterior projection (Ap), which is sometimes called lophi. Ancorae (A) is a pair of projections from the anterior margin. Oval sclerite (not shown) is a small sclerite found at the side of each anterior projection connected by the membrane. Epiphallus is not involved in the gamete transfer. Anterior projections are hooked to female genitalia during copulation to ensure the coitus.

2.1.4. Ovipositor

Although grasshopper ovipositor is not involved in copulation, it is often included as a genital structure (Fig. 2.4). Ovipositor is composed of dorsal (Dv) and ventral valves (Vv) and a pair of inner valve (not shown). At the base of ventral valves, there is a small projection (Lp). Ventral valves are enclosed by basivalvular sclerites, which consist of
upper (not shown), lower (Lbs) and proximal sclerites (Pbs). Internally, there is a pair of lateral apodemes (La) which acts as a muscle attachment site. At the base of lateral apodemes, there is a small sclerite (Ss). Grasshopper ovipositors are modified to dig, and the tips of valves are highly sclerotized.
Figure 2.1: *Schistocerca americana*. Endophallus: A, lateral view; B, ventral view (ejaculatory sac removed); C, dorsal view. Ac: arch of cingulum; Cv: valve of cingulum; Ap: apical valve of cingulum; Fx: flexure; Gpr: gonopore process; Bp: basal valve of penis; Ejs: ejaculatory sac.
Figure 2.2: *Schistocerca americana*. Ectophallus: A, cingulum; B, ectophallic sclerite. Apd: apodemes of cingulum; Zyg: zygoma; Rm: rami of cingulum; Cv: valve of cingulum; Ects: ectophallic sclerite.
Figure 2.3: *Schistocerca americana*. Epiphallus: A, dorsal view; B, ventral view. A: ancorae; B: bridge; Ap: anterior projection
Figure 2.4: *Schistocerca americana*. Ovipositor (ventral view). Dv: dorsal valve of ovipositor; Vv: ventral valve of ovipositor; Lp: external lateral projection of ventral valve; Lbs: lower basivalvular sclerite; Pbs: proximal basivalvular sclerite; Ss: small sclerite; La: lateral apodemes.
2.2. FUNCTIONAL MORPHOLOGY OF MALE GENITALIA

Male genitalia are the intromittent organ to transfer the spermatophore, but the exact process of spermatophore transfer in grasshoppers is still unknown. This is partly due to the difficulty of observing the mating behavior at the muscular level. Genitalia were often described by taxonomists who worked on dried specimens. Taxonomists are mostly interested in the group-specific skeletal variations, and this required removing muscle tissues from the genitalia. However, the muscles are crucial components of genital structures which enable the movement. One of the first descriptions of the phallic muscular system was of *Dissosteira carolina* (Linne) by Snodgrass (1935). His diagram depicted the endophallus filled with muscles. Eades (2000) carefully described the muscular structures in *Roma/ea microptera* (Beauvois) not only in the endophallus, but also in other phallic structures. He was able to identify phallic muscles in several functional categories. As in other muscular systems, the phallic complex requires protractor, retractor, adductor, and abductor muscles. Many layers of muscles are necessary for several reasons. In grasshoppers, males mount on top of females, and bend their abdomens below female genitalia (Alexander, 1967). The subgenital plate is pushed down, and the phallus is exposed. The endophallus contracts to transfer the spermatophore. In order to maintain copulation, several muscles need to work together to ensure the correct position. Other sets of muscles are required to retract genitalia into the body after the copulation. However, because Eades' (2000) study was performed on dead material, the actual movement of muscles was not observed. Recently, Kumashiro and Sakai (2001a) experimentally showed the function of genital muscles in a live cricket.
*Gryllus bimaculatus*. Although *G. bimaculatus* is phylogenetically distant from Acrididae, the general process of muscle movement should be similar between two groups. They demonstrated that the copulation is highly stereotyped and can be described as chain reaction of stimulus and response. Motor neurons innervating phallic muscles exhibit action potentials to enable movements in the phallic complex (Kumashiro and Sakai, 2001b). The function of male genitalia in grasshoppers (and crickets) is to provide muscle attachment sites as specialized apodemes.

Because the muscular apodemes develop in part after molting, the maturation of these structures can be seen only by sequential examination of males of different ages. The present experiment was thus designed to document the daily growth of the genital apodemes by examining freshly molted to sexually mature grasshoppers of known ages. In order to observe the individual variations, multiple specimens of the same ages were studied.
2.3. MATERIALS AND METHODS

2.3.1. Rearing

The experiment was conducted at the United States Department of Agriculture, Agriculture Research Service (USDA ARS) in Sidney, Montana from June 28\textsuperscript{th} to August 1\textsuperscript{st}, 2001. From the colonies reared in the laboratory, the last instars of both male and female \textit{Schistocerca americana} (Orthoptera: Acrididae: Cyrtacanthacridinae) were collected transferred to a clean cage. When the nymphs molted to adults, they were placed in separate tubular cages (approximately 3 inches in diameter and 10 inches in height, with an iron mesh for locusts to rest on) and the date of molting was labeled on the cages. Each cage contained 3 males and 1 female, but when an individual died during the course of experiment, it was immediately removed. Locusts were daily fed fresh Romaine lettuce and wheat bran, and the experiment ran at 30°C, 12:12 Light:Dark cycle. 35 such cages were set up and insects were killed daily by freezing at –20°C for 30 minutes to collect developmental data. After the genitalia were dissected, the whole specimens were stored in 95% EtOH.

Fifteen \textit{Schistocerca gregaria} and fifteen \textit{Locusta migratoria} (5 freshly molted, 5 2-week-old, and 5 sexually mature for each species) were generously provided by Dr. Steve Simpson at University of Oxford.
2.3.2. Dissection and Data collection

Male genitalia were extruded by inserting a probe under supra anal plate after the
 technique described by Hubbell (1932). When the phallic complex was exposed,
 surrounding membranes were carefully removed using forceps and dissecting scissors.
 Female ovipositors were dissected by making a slit at the distal part of abdomen using
 scissors. Surrounding membranes and muscles were carefully removed. Digital
 photographs of genitalia with muscles intact were taken. Brief description of color and
 morphology was recorded each day. Each specimen of genitalia was placed in 10% KOH
 solution for several hours to dissolve muscles. Cleared genitalia were placed in a vial
 filled with glycerin, and each specimen was given an identification number.

Twenty different dimensions of male genitalia were measured using an ocular
 micrometer attached to a microscope (Fig. 2.5, 2.6). Table 2.1 explains the abbreviations
 of the structures measured. Length and width of the lateral apodemes of female
 ovipositors were measured the same way (Fig. 2.7). Genitalia specimens were placed on
 glass beads in order to position them easily. Because the phallic complex was comprised
 of several parts, dimensions of cingulum were measured first and endophallus was
 dissected afterwards.

Drawings of the phallic complex (epiphallus, ectophallic sclerite, cingulum, and
 endophallus) from freshly molted, 14-day-old, 35-day-old specimens were prepared using
 camera lucida. Specifically, the proximal view of epiphallus, the frontal view of
 ectophallic sclerite, the frontal-lateral view of cingulum, and lateral, dorsal, ventral view
 of endophallus were drawn. For endophallus, the endophallic sac was removed for the
dorsal and ventral view. Drawings of the ovipositor were also made. The muscle tissues
were removed, and the ventral view was drawn from freshly molted, 14-day-old, 35-day-old specimens. Drawings for *Schistocerca gregaria* and *Locusta migratoria* were made in the same way.
Figure 2.5: Parts of genitalia measured. A: cingulum; B: ectophallic sclerite; C: epiphallus (dorsal view); D: epiphallus (ventral view)
Figure 2.6: Parts of endophallus measured. A: lateral view; B: ventral view; C: dorsal view.
Figure 2.7: Parts of ovipositor measured. LaL: length of lateral apodeme; LaW: width of lateral apodeme

<table>
<thead>
<tr>
<th>Ac1</th>
<th>length of arch of cingulum (lateral)</th>
<th>EAp</th>
<th>length of anterior projection of epiphalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac2</td>
<td>length of arch of cingulum (lateral)</td>
<td>EcC</td>
<td>width of midprojection of ectophallic sclerite</td>
</tr>
<tr>
<td>Ac3</td>
<td>width of arch of cingulum (dorsal)</td>
<td>EcH</td>
<td>width of half of ectophallic sclerite</td>
</tr>
<tr>
<td>Ac4</td>
<td>width of arch of cingulum (dorsal)</td>
<td>EpB</td>
<td>width of bulbous base of epiphalus</td>
</tr>
<tr>
<td>Ap</td>
<td>length of apical valve of penis</td>
<td>EpW</td>
<td>horizontal width of epiphalus</td>
</tr>
<tr>
<td>ApdR</td>
<td>width of apodeme ridge of cingulum</td>
<td>Fx</td>
<td>width of flexure</td>
</tr>
<tr>
<td>ApW</td>
<td>width of apical valve of penis</td>
<td>Fx+Bp</td>
<td>length of flexure + basal valve of penis</td>
</tr>
<tr>
<td>Ar</td>
<td>width of arms to apical valve</td>
<td>Gpr</td>
<td>width of gonopore process</td>
</tr>
<tr>
<td>B</td>
<td>width of bridge of epiphalus</td>
<td>LaL</td>
<td>length of lateral apodeme of ovipositor</td>
</tr>
<tr>
<td>Bp</td>
<td>width of half of basal valve of penis</td>
<td>LaW</td>
<td>width of lateral apodeme of ovipositor</td>
</tr>
<tr>
<td>CW</td>
<td>width of cingulum</td>
<td>RmW</td>
<td>width of rami of cingulum</td>
</tr>
</tbody>
</table>

Table 2.1: Abbreviations of parts measured from *Schistocerca americana* phallic complex and ovipositor.
2.3.3. Statistical Analysis

All measurements were logarithmically transformed to normalize and plotted against the days after emergence using MINITAB. Since all grasshoppers were sexually mature by day 30 after emergence, the data points between day 30 to 35 formed a straight line. The average of measurements from the last five days was arbitrarily assumed to be fully formed, and each data point was divided by this value. The resulting number is the percent development. Then, the first day of reaching 90% development for each part was determined. There was a small number of individuals with the slower maturation, and thus the last day of reaching 90% development from at least one individual was recorded as well.
2.4. RESULTS

2.4.1. Developmental sequence of the phallic complex

The following descriptions are the observations that I made immediately after dissection. Although most locusts follow the similar patterns, some seemed to develop slower than others. For each day, I described the new structures or patterns that I did not observe previously. Female ovipositors did not change externally except for muscle mass inside, but internally lateral apodemes muscles grew through time. However, because the observation was made before muscle tissues were removed, I could not document the cuticle deposition of ovipositor. Day 1 indicates the first day after the emergence.

Day 0-1: All the structures were not sclerotized. The chitin had not been deposited thoroughly and the color of the phallic complex was opaque and white. The epiphallus was fully formed but the thickness of cuticle was very thin and was white in color. The cingulum, ectopallic sclerite, and endophallus were not fully formed and very fragile-looking. The arch of cingulum in the endophallus was absent.

Day 2: All the parts started to be sclerotized and the structures turned light brown in color. Trachea was found within the phallus. In the cingulum, the lateral parts of rami were white whereas others parts were all brown.

Day 3: The white areas closed to rami began to be filled with cuticle.

Day 4: All the structures were more robust due to cuticle deposition. In the endophallus, the distal projection of the arch of cingulum started to develop. It was very thin and cylindrical, and appeared to originate from both below the zygoma and the base of arch of
cingulum. This phenomenon only happened in one male. But, by day 5, the structure
appeared in all males.

**Day 6-7:** More cuticle deposition was in process, but not much different from the day 5.
It seemed as though certain individuals developed faster that others because the amount of
cuticle deposition was different for each individual.

**Day 8-9:** The arch of cingulum became thicker and larger.

**Day 10:** Cuticles became more rigid and the anterior projection of epiphallus was
hardened.

**Day 11:** The anterior projection of epiphallus began to have double layers internally. The
hour-glass shaped portion below zygoma became smaller. Arch of cingulum was getting
thicker, but still a cylindrical shape.

**Day 12-13:** The arch of cingulum became thicker, and other parts became more robust.

**Day 14-16:** Tip of anterior projection of the epiphallus became darker, and the hour-glass
shaped portion below zygoma became even smaller.

**Day 17-20:** The morphology of the phallus was similar to the fully mature ones. Arch of
cingulum became bulbous. But external coloration of locusts was brown indicating sexual
immaturity.

**Day 21:** Some locusts turned bright yellow indicating sexual maturity. From day 21 to
day 30, most locusts turned yellow. On day 30, there were still males with brown
coloration. Sometimes copulation and oviposition were observed. The phallus was fully
matured.

**Day 31-35:** All the locusts were bright yellow and the phallic complex was highly
sclerotized and mature.
2.4.2. Rate of development

Different structures of genitalia had different rates of development. Overall size of epiphallus, ectophallic sclerite, and cingulum did not change over time whereas the size of endophallus dramatically changed over time. However, even within the non-changing structures, cuticle deposition seemed to happen over time because the structures became more rigid as time passed. Especially, the width of apodemes of cingulum (ApdR) and bulbous bases of epiphallus (EpB) increased. All the parts measured from endophallus develop to a certain extent except for the length of apical valve of penis (Ap).

The overall size of cingulum did not change. Figure 2.8 shows the developmental patterns of parts measured in cingulum. Width of cingulum (CW) as well as width of rami (RmW) remained constant. However, the width of apodemes of cingulum (ApdR) changed dramatically. The pattern of development is similar to the bulbous based of epiphallus. Yet, the cuticle deposition slowed down around day 14 after emergence. Figure 2.10A shows the overall changes of cingulum.

Ectophallic sclerite also did not change in overall size (Fig. 2.9). Both the width of half of ectophallic sclerite (EcH) and width of midprojection (EcC) remained constant (Fig. 2.10B). However, similar to cingulum, the thickness of cuticle increased which was not measured.

Figure 2.11 shows the developmental patterns of four dimensions measured in epiphallus. Overall size of epiphallus (EpW) including bridge (B) and anterior projection (Ap) remained the same from the emergence. The thickness of cuticle increased but not measured. The most striking pattern is from the bulbous bases of epiphallus (EpB). Right after the emergence, there was no bulbousness because it was the result to cuticle
deposition. The cuticle seemed to be deposited proportionally everyday until day 26 and after that no more deposition occurred. Figure 17A is the graphical representation of the developmental changes.

Endophallus was significantly different in the developmental patterns comparing to other components of the phallic complex. Both overall size and width changed, and the projection of arch of cingulum was not even present after emergence. Only non-changing part was the length of apical valve of penis. Figure 2.12 shows the dorso-ventral growth of endophallus. Although both structures grew, the width of flexure (Fx) increased less than the width of gonopore process (Gpr) (Fig. 2.17B). Both structures ceased to develop around the similar time period (10 days after emergence). Endophallus also developed laterally (Fig. 2.13). Width of arms to apical valve (Ar) was the least to change ceasing to develop after 5 days. Width of apical valve (ApW) and width of basal valve of penis (Bp) both grew in a similar pattern. They ceased to develop after 10~14 days after emergence.

Interesting pattern can be found in length-wise development (Fig. 2.14). The length of apical valve of penis remained the same whereas the length of flexure and basal valve of penis combined changed over time. In fact, the distal part of endophallus (where apical valve of penis is located) remained the same, and the proximal part elongated through cuticle deposition (Fig. 2.17C). Arch of cingulum showed the most peculiar developmental patterns. The distal projection of arch of cingulum was absent for the first three days after emergence, and it suddenly formed on day 4. It started as a thin cylindrical structure and developed into a bulbous structure. Figure 2.15 shows that the length of the distal projection (Ac1) stayed the same after it appeared, but the thickness (Ac2) continued to increase until about 20 days after emergence. Thickness of the distal
projection (Ac3) also increased laterally (Fig. 2.16). However, the width of base of arch of cingulum (Ac4) ceased to increase after about 5 days after emergence (Fig. 2.16). It should be noted that the ejaculatory sac was present immediately after the emergence although the cellular maturity was not measured.

In females, the lateral apodemes of ovipositor continued to develop after emergence (Fig. 2.19). The external valves did not change in size except that the muscle mass inside valves increased. Figure 2.17 shows that both length and width increased over time. Interestingly, the length of lateral apodemes (LaL) seemed to develop faster. The width of lateral apodemes (LaW) continued to increase until sexual maturity. Since the experiment was designed to measure the development of male genitalia, I did not have the complete data for females. However, the data from only 32 specimens were enough to show the pattern of development.

Table 2.2 summarizes the days after emergence where at least one individual showed the 90% of full growth. Because individuals had different rates of development, certain ones developed slower than others. Thus, the last day of an individual with still less than 90% of full growth was recorded. In the phallic complex, most parts formed 90% of full growth by two weeks after molting. The exceptions were the bulbous base of epiphallus (EpB, 26 days) and the width of distal projection of arch of cingulum (Ac2, 19 days). In ovipositor, the length of lateral apodemes (LaL) reached 90% of full growth in 13 days, whereas the width (LaW) increased for one more week (20 days).
Although most individuals matured at the similar rate, a small proportion of individuals simply did not mature fast enough. Bright yellow coloration is the indication of sexual maturity, which all individuals from day 31-35 showed, but internally certain parts of genitalia did not reach the full growth.
Figure 2.8: Development of Cingulum. Circle: ApdR; Square: CW; Triangle: RmW
Figure 2.9: Development of Ectophallic Sclerite. White: EcH; Solid: EcC
Figure 2.10: Development of phallic complex. A, cingulum; B, ectophallic sclerite
Figure 2.11: Development of Epiphallus. Circle: EpW; Square: EpB; Diamond: EAp; Triangle: B.
Figure 2.12: Development of Endophallus (dorso-ventral). White: Fx; Solid: Gpr
Figure 2.13: Development of Endophallus (lateral). Circle: Ar; Square: ApW; Triangle: Bp
Figure 2.14: Development of Endophallus (length-wise). White: Ap; Solid: Fx+Bp
Figure 2.15: Development of Endophallus (Arch of cingulum). White: Ac1; Solid: Ac2
Figure 2.16: Development of Endophallus (Arch of cingulum). White: Ac3; Solid: Ac4
Figure 2.17: Development of phallic complex. A, epiphallus; B, endophallus (lateral); C, endophallus (ventral). (In C, endophallic sac was removed for 2 weeks old and sexually mature specimens)
Figure 2.18: Development of lateral apodemes of ovipositor. White: length; Solid: width
Figure 2.19: Development of ovipositor.
<table>
<thead>
<tr>
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<tr>
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</tr>
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<td>EAp</td>
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<td>Not Changing</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Not Changing</td>
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<td></td>
</tr>
<tr>
<td>Ectopallic Sclerite</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>31</td>
<td></td>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
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</tr>
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</tr>
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<td>30</td>
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</tr>
<tr>
<td>Fx+Bp</td>
<td>10</td>
<td>35</td>
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</tr>
<tr>
<td>Ovipositor</td>
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<td></td>
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</tr>
<tr>
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<tr>
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</tr>
</tbody>
</table>

Table 2.2: Rate of development in each part measure from *Schistocerca americana* phallic complex and ovipositor. Third column indicates the first day when at least one individual with 90% of full growth appeared. The fourth column indicates the last day when an individual was still underdeveloped below 90% of full growth. Not changing means the parts measured remained constant for 35 days.
2.4.3. Post-emergence development of male genitalia in other species

In order to see if the genitalic development is widespread in grasshoppers, I examined a closely related species, *Schistocerca gregaria*, and a distantly related species, *Locusta migratoria*. The phallic complexes of *S. americana* and *S. gregaria* were very similar, but the phallic complex of *L. migratoria* was drastically different from *Schistocerca*. The size of the whole insect was similar between *Schistocerca* and *Locusta*, but *L. migratoria* had a phallic complex three times as big as that of *Schistocerca*. Furthermore, it was highly sclerotized immediately after emergence, indicated by the brown color of cuticle.

Although I did not have daily data for these two species, the specimens of comparable ages were available (freshly molted, 2 weeks after molting, and 35 days after molting). Because these time intervals were rather widespread, the exact sequence of development was difficult to determine. However, a pattern arose when the same structures from three species were compared.

Figure 2.20 shows the development of cingulum in three species. In all cases, the apodemes of cingulum increased in width and length. Also, the distal portion of the cingulum remained relatively unchanged whereas the cuticle deposition of the proximal portion was dramatic. Compared to *S. americana*, the cingulum of *S. gregaria* was more fragile. Unlike *S. americana* and *S. gregaria*, the whole phallic complex of *L. migratoria* was highly sclerotized after emergence (Fig. 2.20C).

The similar pattern found in *S. americana* ectophallic sclerite was found in two other species (Fig. 2.21). The overall size of the structure remained constant and the thickness of cuticle increased. Likewise, epiphallus had the similar patterns of
development where the overall size remained constant (Fig. 2.22). In both *Schistocerca* species, the bulbous base of epiphallus increased in width through time. The drawing of freshly molted *S. gregaria* (Fig. 2.22B first drawing) has a smaller anterior projection of epiphallus, but this is an artifact that reflects the fragile nature of the structure. In *L. migratoria*, no structure behaved like the bulbous base in *Schistocerca*, but the thickness of cuticle increased through time (Fig. 2.22C).

The most structural changes could be found in endophallus (Fig. 2.23). In both *Schistocerca* species, the arch of cingulum was absent immediately after emergence and developed into a cylindrical shape to a bulbous shape (Fig. 2.23A, B). Basal valve of penis and gonopore process both increased in width and length. There seemed to be no equivalent structure to the arch of cingulum in *L. migratoria*. The apical valve of penis remained constant, but the basal valve of penis went through dramatic structural changes (Fig. 2.23C).
Figure 2.20: Development of cingulum. A, *Schistocerca americana*; B, *Schistocerca gregaria*; C, *Locusta migratoria*. Drawings of *L. migratoria* were reduced 2x to fit to figure.
Figure 2.21: Development of ectophallic sclerite. A, *Schistocerca americana*; B, *Schistocerca gregaria*; C, *Locusta migratoria*. Drawings of *L. migratoria* were reduced 2x to fit to figure.
Figure 2.22: Development of epiphallus. A, *Schistocerca americana* (ventral view); B, *Schistocerca gregaria* (ventral view); C, *Locusta migratoria* (dorsal view). Drawings of *L. migratoria* were reduced 2x to fit to figure.
A. *Schistocerca americana*

B. *Schistocerca gregaria*

C. *Locusta migratoria*

Figure 2.23: Development of endophallus (lateral view). A, *Schistocerca americana*; B, *Schistocerca gregaria*; C, *Locusta migratoria*. Drawings of *L. migratoria* were reduced 2x to fit to figure.
2.5. DISCUSSION

2.5.1 Development of genitalia and benefit of delayed maturation

The function of male genitalia is undoubtedly the copulation, but this is rather an over-simplified statement. The present study revealed that each component of the phallic complex experiences a structural change that seems to be closely associated with the muscles responsible for the movement during copulation. In essence, the male genitalia are the complex structure composed of several internal apodemes to make copulation possible. The growth of apodemes is accompanied by the increase in muscle mass. Though not quantified, I was able to observe the significant muscle increase in both male genitalia and lateral apodemes of ovipositor in *Schistocerca americana*. The similar patterns could be found in thorax where thoracic apodemes grow in dimension as the flight muscles increase in mass (Neville, 1963b).

For example, the elaborated structures in the proximal portion of the endophallus probably correspond to the muscles that enable the spermatophore transfer. Bulbous base of epiphallus is perhaps the attachment site of muscles responsible for stably holding females during copulation. Apodemes of cingulum are likely to provide attachment site for the muscles to extrude the phallic complex during copulation. Similar reasoning applies to the ovipositor as well. The lateral apodemes of ovipositor provide the muscle attachment sites to enable the ovipositor valves to move and dig for the oviposition. Elastic nature and cuticle deposition patterns all indicate that the male genitalia and the lateral apodemes of ovipositor are resilin based. In fact, several layers of chitin can be observed in the basal valve of penis, which look similar to chitin deposition in thoracic
apodemes (Neville, 1963b). Freshly molted specimens, however, do not have these apodemes. The cuticle deposition has yet to occur and muscle cells have not differentiated. During the course of the sexual maturation, these changes occur. Therefore, I argue that the male genitalia are functionally incapable of copulation during maturation because the apodemes to support the muscle movement have not yet developed. Similarly, I argue that the ovipositor is functionally incapable of ovipositing during sexual maturation. The timing of the complete development of genitalia and the timing of sexual maturity coincide, which demonstrates that there is a considerable time in the grasshopper adult life when individuals are functionally incapable of mating.

Despite these cuticular changes, male genitalia are more than internal apodemes. Each component of the phallic complex also had non-changing portions. Epiphallus, ectophallic sclerite, the distal portion of cingulum, and the distal portion of endophallus are these. These parts were not associated with muscles, and the only changes were of thickness of cuticles. Moreover, these non-changing structures have a specific function other than the movement. For example, the anterior projections of epiphallus, which do not change in size, are directly involved in holding the female during copulation. Apical valve of penis is the portion that penetrates to the bursa copulatrix of the female. Distal portion of cingulum directly touches the female counter-part during copulation. Hence, all the non-changing structures seem to be in direct contact with females during copulation. If the function of male genitalia is the internal courtship as proposed by Eberhard (1985), it makes sense for the courtship-related structures to be relatively stable. Male genitalia are thus composed of two functionally different categories: the structures responsible for the movement (apodemes) and the structures responsible for the internal
courtship. This pattern of both development and non-development was found in *S. gregaria* and *L. migratoria*, which suggests that the binary function of male genitalia can be widespread.

Life history theory states that if the benefit of greater longevity outweighs the benefit of earlier reproduction, it is more advantageous for an organism to delay reproduction (Stearns, 1992; Roff, 1992). Both *Schistocerca* and *Locusta* are strong fliers with a migrating behavior (Uvarov, 1966). These insects form a swarm that has its own unique dynamics. Therefore, it is conceivable that the flight is the most important aspect to ensure survival of the insect. If this is true, there is every benefit for the early maturation of structures associated with the flight, namely flight muscle and thoracic apodemes. Weis-Fogh (1952) showed that the full flight capacity is achieved in the early adult stage of *S. gregaria*, and Neville (1963b) showed that the thoracic apodemes associated with the flight also completes the development in one week after emergence. Flight, while important for the swarm dynamics, is also important in terms of avoiding predators and locating food. Solitary *Schistocerca* do not swarm, but they do migrate (Steedman, 1990). Thus, the early maturation of the flight-related structures is essential in the grasshopper's survival.

The present study is the first to show that the genitalia change structurally during sexual maturation, and this development is accompanied by the cuticle deposition and the muscle development. Sexually immature adults go through an intensive feeding period (Norris, 1954). The food they consume is an energy source for the migration, but it is likely that it fuels the cellular differentiation. In other words, the nutrients insects obtain during the intensive feeding are essential for the development of cuticle and muscle. This
therefore constitutes a limited resource pool for maturation of two independent structures: flight-related structures and reproduction-related structures. Suppose insects allocated the resource according to the best life history strategy. Because the flight is so much important for the survival of an insect, it is possible that the resources are utilized to develop the flight-related structures first. However, this is only a half the story because the life history theory dictates the trade-off between two life history traits (Zera and Harshman, 2001). Then what is the benefit of reproduction? The obvious answer is to pass on the genes to the next generation, which is the goal of the adult insects. Yet, the cost of reproduction, especially in female grasshoppers, is extremely high. The nature of oviposition in grasshoppers includes the stretching of abdominal membranes and the egg pod formation. During the oviposition, females are the most vulnerable and the recovery takes a considerable time (Norris, 1952). Thus, the benefit of developing flight-related structures outweighs the benefit of the reproduction.

Then, why do males have a delayed maturation? It should be noted that comparing to females, the males mature late. Obviously it would be beneficial for both sexes to synchronize the age at maturity. However, there is a differential cost of reproduction in each sex. Males only need to copulate and transfer spermatophore, but females have to go through a physical labor of oviposition. In fact, copulation does not necessarily ensure the fertilization (Norris, 1954; Eberhard, 1985). Therefore, it is beneficial for females to choose the males good enough to commit oviposition. Given, that the longevity of Schistocerca is over two months, females can possibly endure extra few more days to mate with the fittest male.
However, what if there is another mean to ensure survival? In many cases, brachypterous grasshoppers such as *Dactylotum bicolor* (painted grasshopper) have aposematic coloration and toxic chemicals. Obviously they lack the flight capacity, which was the most important method for survival in *Schistocerca* and *Locusta*. Is there a trade-off in this case? Since being aposematic and unpalatable compounds the model, let us assume that there is a hypothetical brachypterous grasshopper that is neither aposematic nor unpalatable. If this brachypterous grasshopper has a shorter maturation, it is possible to hypothesize that the benefit of reproduction outweighs the benefit of survival. However, if this imaginary grasshopper indeed has a long maturation period, one might suspect that the long maturation period is intrinsic to grasshoppers. In this case, the life history theory explaining the age at maturity is not supported. If this brachypterous grasshopper has a protection, such as toxicity, the maturation period may be different according to the theory. For example, if the maturation period is long, the benefit of reproduction is outweighed by the benefit of survival, in this case probably eating toxic plants. If however the maturation period is short, again the theory loses its support. This reciprocal relationship is worth testing, but no study has yet to test this idea.

One of the weaknesses in applying the life history theory in grasshopper sexual maturation is revealed when explaining the life history of nymphs. I have argued above that the flight is the most important trait for the survival of a grasshopper, but nymphs obviously lack the flight capacity. Nymphs also do not have a cost of reproduction. Therefore, the trade-off between survival and reproduction is not applicable to the nymphal stage. *Schistocerca* nymphs are capable of feeding on toxic plants and develop the aposematic coloration (Sword and Dopman, 1999). Even though nymphs lack the
flight capacity, this unpalatability may protect them from the predators, and there may be costs associated with the evolution of host specificity. However, all these are mere speculations, and finding an adaptive reasoning to the physiological phenomenon such as delayed sexual maturation is a difficult matter. It may indeed that all hemimetabolous insects are intrinsically slow-maturing. Life history theory, however, seems to explain the present data the best, and more empirical studies are needed to test the hypothesis.

2.5.2. Similarities and differences in developmental patterns

Despite the phylogenetic distance (Flook and Rowell, 1997), the male genitalia of *Schistocerca* and *Locusta* follow the similar developmental patterns. Epiphallus, ectophallic sclerite, and cingulum remain relatively constant in size, and the proximal portion of endophallus experience dramatic structural changes. In other words, the homologous structures in two phylogenetically distant groups have the same pattern of development although the morphologies of two groups are very different. In a broad sense, the developmental patterns of male genitalia could be a synapomorphic character for two groups. Of course, a pattern is not a discrete character, but this finding leads to some interesting speculations in terms of evolution of male genitalia in suborder Caelifera (Orthoptera). Dirsh (1956) studied the phallic complex of all the families in Caelifera and proposed the following sequences shown in Table 2.3.
Both *Schistocerca* and *Locusta* belong to the superfamily Acridoidea and have the most advanced form of a phallic complex, but the primitive groups such as Eumastacoidea do not have the ectophallus. Given that the phallic complex is derived from ectoderms (Dirsh, 1973), the absence of the structures in the primitive groups could be explained by no differentiation. In other words, the more differentiation in more advanced groups is in a sense the result of heterochrony. No one knows the maturation period of Eumastacoidea, but if it is considerably shorter than that of *Schistocerca* and *Locusta*, the sclerotized structures in Acridoidea is the hypermorphosis.

Although both *Schistocerca* and *Locusta* have a similar swarming biology, *L. migratoria* has a shorter maturation period (two weeks), and the male genitalia are highly sclerotized after the final emergence unlike *Schistocerca*. In both genera, the cuticles are continuously deposited similar, but the morphological differences of male genitalia between final emergence and sexual maturity in *L. migratoria* are not as great (Fig. 20-23). Moreover, the male genitalia of *L. migratoria* are about three times as big as those of

<table>
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<td>Ectophallic sclerite</td>
<td>Absent → Present</td>
</tr>
<tr>
<td>Endophallus</td>
<td>Arch-shaped → Rod-shaped → Flexured</td>
</tr>
</tbody>
</table>

Table 2.3: Evolutionary changes of phallic complex in Caelifera (modified from Dirsh (1956))
S. americana and S. gregaria. Thus, although the patterns of development could be similar, the duration and the rate of cuticle deposition could vary. The higher phylogenetic relationships among the subfamilies of Acrididae are not well understood, but the differences in maturation period and sclerotization may be the result of heterochrony as well. Comparing to L. migratoria, Schistocerca has a retarded maturation rate, which is equivalent to hypermorphosis. If L. migratoria is a more advanced species, the shorter maturation period with more sclerotization is the result of acceleration.

2.5.3. Taxonomic implication of genitalia development

Most taxonomic studies deal with museum specimens in which researchers do not know the exact ages of the specimens. Although there is a method to estimate the age based on the age ring in cuticles (Neville, 1963a), it is rarely used in taxonomy. Adult grasshoppers look externally identical regardless of their ages, the present study demonstrate that sexually immature grasshoppers have less developed genitalia whose morphology is significantly different from that of the sexually mature ones. If a species is defined using the less developed genitalia, a later taxonomist may define the same species as a different one based on the fully developed genitalia. In fact, Dirsh (1974: 166) defined a new species, Schistocerca braziliensis, based on immature male genitalia without knowing the developmental aspect. His drawing of endophallus clearly shows the characteristic of undeveloped arch of cingulum. It is also dangerous to define a species based on the length and width of apodemes. The present study demonstrates that the lateral apodemes of ovipositor change in length and width. This reasoning probably
applies to most internal apodemes. Unlike external characters, internal apodemes need to grow in order to provide the attachment site for growing muscles. Thus, it would be misleading to use such characters without knowing the developmental stage a specimen is in.
Schistocerca (Stål) (Orthoptera: Acrididae: Cyrtacanthacridinae) is the most enigmatic genus of grasshoppers, known for the swarming behavior and the peculiar biogeographical distribution. However, the taxonomy and the phylogeny are very poorly understood despite the agricultural importance.

In this chapter, a phylogeny of North American Schistocerca is proposed for the first time based on a cladistic analysis using morphological characters. The taxonomic history and the swarming behavior are discussed, and the possible scenario of the current biogeographical distribution is proposed in light of the phylogeny.
3.1. TAXONOMIC REVIEW

The genus *Schistocerca* is known to be very difficult to classify because of the similarities among species (Hubbell, 1960). Morphometric studies, cuticular hydrocarbon studies, and hybridization studies all failed to produce a plausible classification for the whole genus (Dirsh, 1974; Grunshaw *et al.*, 1990; Harvey, 1981). However, this genus is not unfamiliar to the scientific world as well as the general public because of the infamous desert locust recorded in Bible, Koran, and many other ancient literatures (Dirsh, 1974). The desert locust, *S. gregaria*, is still one of the most damaging locusts in Africa and Middle East. Because of its agricultural importance, there is a great deal of studies on biology, physiology, ecology, behavior, and population dynamics of *S. gregaria* (Uvarov, 1966, 1977), but the biological information on other species is surprisingly lacking.

Currently, there is no solid taxonomic work on *Schistocerca*. From the time when Stål erected the genus in 1873, nearly 200 species names were attributed to it. Many of them were synonyms, and some of them even belong to other genera now (Dirsh, 1974). In 1899, Scudder published the first comprehensive revision, in which he recognized 42 species. However, many species were described based on color characters from single specimens (Scudder, 1899) and the revision was not very useful to the taxonomists (Hubbell, 1960; Dirsh, 1974). In 1960, Hubbell presented a partial revision of North American *alutacea* group, based on genitalia structures and morphometrics. Even though he admitted that he had a difficulty differentiating among *S. alutacea*, *S. rubiginosa*, and *S. lineata* due to extreme similarities, Hubbell (1960) carefully identified the distinct male genitalia characters and concluded that three species belonged to the same taxonomic
group. The most recent complete revision by Dirsh (1974) included 22 species with numerous subspecies including six new species and four new subspecies. Dirsh (1974) exclusively used the morphometrics to define a species, and when the characters, such as ratios between cranium length and femur length, overlapped among species, he lowered the species status to subspecies, or in a worse case, synonymized many species in to one. In addition, Hubbell’s (1960) careful study was ignored, resulting in several subspecies within *S. alutacea* (Harvey, 1981). One of the most dramatic things Dirsh did was to synonymize *S. gregaria* under *S. americana*, creating a subspecies called *Schistocerca americana gregaria*. He was correct in identifying affinities between two species, but his approach was rather radical. Furthermore, Dirsh (1974) used no biological information other than distribution, and this cast doubts among many orthopterists.

In order to test Dirsh’s (1974) classification, Harvey (1979, 1982) did several hybridization studies with subspecies in the *americana* complex, which included *S. americana*, *S. gregaria*, and South American locusts *S. cancellata* and *S. piceifrons*. He determined that several subspecies were actually reproductively isolated from each other and thus deserved to be separate species under the biological species concept. In separate studies using hybridization techniques, Jago *et al.* (1979, 1982) agreed the species status of the subspecies in the *americana* complex. In 1981, Harvey published a partial revision of the *americana* complex and concluded that there are six closely related species in the complex.

No study has focused on the taxonomy of sedentary North American *Schistocerca* since Dirsh’s revision. Although many orthopterists consider the subspecies of *S. alutacea* as valid species, the current taxonomic status stands unchanged. There are
currently ten *Schistocerca* species found in North America, and of these eight species are truly sedentary with no swarming behavior. *Schistocerca americana*, which is distributed from Midwest to Southeast U.S., is considered to be a close relative to South American locusts (Harvey, 1981), and is perhaps a result of northward speciation. *Schistocerca nitens* which occurs in Midwest regions has an extremely wide distribution down to Brazil, and is perhaps not a natural species. Sedentary *Schistocerca* includes *S. damnifica*, *S. ceratiola*, *S. obscura*, and six subspecies of the *alutacea* complex *sensu* Dirsh. Of these, *S. lineata* is polymorphic depending on the host plants where individuals feeding on a toxic *Ptelea* have aposromatic coloration (Sword and Dopman, 1999). This black and yellow aposromatic coloration remotely resembles the coloration of *S. albolineata*, and Dirsh (1974), without knowing the ecology, commented that aposromatic specimens are the intermediate between two species.

As mentioned above, *Schistocerca nitens* is the most problematic species in terms of classification. Beside its wide distribution, Brazilian specimens are morphologically very different from North American specimens. Dirsh (1974) synonymized 16 species under *S. nitens* in his revision only based on measurements. There is a possibility that this species can be divided into several valid species.

Finally, Dirsh (1974) also created several new species mostly based on the male genitalia morphology. Dirsh was very active in using male genitalia as taxonomic characters (Dirsh, 1973), and in fact he described the male genitalia of all the families and subfamilies in Caelifera (Dirsh, 1956). Although he was a major proponent of the male genitalia use in taxonomy, he did not know the developmental nature of the male genitalia, which has been discussed in depth in my previous chapters. For example, a new
species, *S. braziliensis*, was described based on the immature male genitalia according to his drawings (Dirsh, 1974: 166).

In essence, because of the excessive use of morphometrics and the poor understanding of male genitalia development along with the lack of ecological knowledge in Dirsh's revision, *Schistocerca* still remains as a taxonomically problematic group.
3.2. BIOGEOGRAPHY

*Schistocerca* has a very peculiar biogeographical distribution, which caused various speculations about the origin of the genus. Within the genus, only one species occurs in the Old World, mainly Africa and Middle East, and all the other species occur in the New World. Cyrtacanthacridinae, which *Schistocerca* belongs to, is the Old World subfamily except for one Galapagos genus, *Halmenus*. Therefore, the main question about the current distribution concerns how the genus diversified (Amedegnato, 1993; Dirsh, 1974; Grunshaw et al., 1990, Waloff and Pedgley, 1986).

The modern grasshoppers (Acrididae) are considered to be diversified in the Tertiary period (Amedegnato, 1993). They originated from the Old World, but the South American subfamilies seem to have resulted from the Cenozoic vicariance event. A recent molecular study by Rowell and Flook (1998) determined that the evolution of the Acridoidea seemed to be the product of a single explosive radiation, very possibly coinciding with the spread of the angiosperms in the Cretaceous. Thus, although Orthopteroid insects are primitive within the class Insecta, the modern grasshoppers have a relatively recent origin. The subfamily Cyrtacanthacridinae is ecologically arboreal, and the groups that are connected with the herbaceous vegetation are considered to be the result of later adaptation (Dirsh, 1974). This indicates that the time of diversification for the subfamily is Eocene or Oligocene when the arboreal vegetation was predominant on the earth. At this time, South American and Africa were already separated by the Atlantic Ocean, and given that the subfamily has its origin in Africa, some form of migration event has to be considered to explain the current distribution. In 1988, a large swarm of *S.*
gregaria flying from the western coast of Africa across the Atlantic Ocean was observed (Ritchie and Pedgley, 1989). This observation allowed the possibility that the locusts could have had the ability to fly a long distance to invade the New World or vice versa.

There is no debate about the fact that the ancestral Schistocerca originated from the Old World because of the distribution of Cyrtacanthacridinae. However, much debate has been devoted to the question that if S. gregaria is the ancestor of the genus. Historically there have been two competing hypotheses on the origin of Schistocerca.

The Old World origin hypothesis assumes that the S. gregaria-like ancestor has remained unchanged (thus explains the presence of S. gregaria in the Old World) and it originated from the Old World and later populated the American continent, and found unoccupied ecological niches and rapidly produced an explosive adaptive radiation (Dirsh, 1974).

The New World origin hypothesis assumes the S. gregaria invading the Old World after the genus diversified in the New World. In his revision, Dirsh (1974) speculated that the ancestral S. gregaria originating from the New World colonized the Old World, either by crossing the Bering land bridge during the Tertiary period or by flying over the Atlantic. As a result, he made this species as a subspecies of S. americana, and suggested that the species in the americana complex are the most primitive form in the genus. He also mentioned that because there is no cyrtacanthacridine genus in the Old World with which Schistocerca has close affinity, the separation between Schistocerca and other cyrtacanthacridine genera is old (Dirsh, 1969). However, both hypotheses have problems in explaining the current distribution.

If Schistocerca gregaria (or S. gregaria-like ancestor) were the basal species and gave rise to the New World species, then it is curious that there is only a single species
across the ancestral range whereas there are many in the newly colonized area. Harvey (1979, 1981, 1982) and Jago et al. (1979, 1982) demonstrated that the *S. gregaria* has a close affinity to the *americana* complex, which suggests that the species in this complex also have not changed much since the colonization. The notion of unchanging ancestor is difficult to comprehend. The more plausible explanation is *S. gregaria* migrating back to Africa from the American continent. This idea is, however, difficult to assume because of the prevailing wind from the Old World to the New World today. Yet, the wind direction at the time of *Schistocerca* diversification might have been different from that of today. Another problem lies in the origin of *Halmenus*. This brachypterous genus contains four species, all endemic to the Galapagos Islands. Given that the Galapagos Islands are only about two million years old, the age of *Halmenus* is less than two million years old. Amedegnato (1993) suspected the insular brachypterism, which indicates that the ancestor of *Halmenus* migrated to the islands and lost their wings subsequently.

The importance of *Halmenus* is that it is only other cyrtacanthacridine genus in the New World (Amedegnato (1993) claimed that there is another cyrtacanthacridine genus in Cuba, but no male has been described to test this idea). Because of the similarity of genitalia morphology, Dirsh (1974) suggested that *Halmenus* is the relic of the ancestral stock of *Schistocerca*. The idea of *Schistocerca* originating from the Galapagos Islands is also highly improbable. Indeed, the genitalia morphology of two groups is almost identical, but the external structures such as male cercus and subgenital plate of *Halmenus* resemble more to the African relatives (personal observation). This may suggest that the common ancestor of *Halmenus* and *Schistocerca* colonized the American continent less than two million years ago. The relative homogeneity of the phallic structure in
*Schistocerca* indicates that the genus is still very young because other genera in cyrtacanthacridinae have distinct species-specific genitalia morphology (Dirsh, 1974).
3.3. SWARMING BEHAVIOR AND PHASE POLYMORPHISM

The swarming behavior is widespread in many insect groups (for example, honeybee swarm, ladybug hibernation swarm, and locust swarm), and the word "swarm" is very loosely defined. In Acrididae, however, the swarming behavior is often very dramatic, and the species that swarm are specifically called locusts. The locust swarm occurs in extremely large numbers. For example, there can be at least 40 million and sometimes as many as 80 million individuals in each square kilometer of swarm. Half a million locusts weigh approximately one ton, and one ton of locusts eats as much food in one day as about 10 elephants or 25 camels, or 2500 people (Steedman, 1990). Locust plagues occur in some of the world’s most famine-prone areas and, thus, generate considerable concern within both economic and humanitarian contexts (Pener and Yerushalmi, 1998; Showler, 1995).

There are several swarming locusts in Acrididae. *Schistocerca, Locusta, Nomadacris, Locusta, Locustana, Calliptamus, Dociostaurus* and many others in several subfamilies show the swarming behavior (Steedman, 1990; Uvarov, 1966). While other genera are monotypic or only contain one swarming species, *Schistocerca* is unique in that it contains multiple swarming species. These include *S. gregaria* (Africa), *S. piceifrons* (Central America), *S. cancellata* (South America), and *S. interrata* (Peru) (Harvey, 1981; Duranton et al., 2001). *Schistocerca interrata* was described in 1899 and was known to be non-swarming. Only recently, the swarming population of this species was discovered in Peru (Duranton et al., 2001). Swarming locusts have a wide range of food choice, ability to consume great quantities of food, and the ability to multiply their
population exponentially (Steedman, 1990). While *Schistocerca* is most famous for the desert locust, *S. gregaria*, relatively few studies concentrate on other three outbreak species.

Swarming behavior is a complex response to both biotic and abiotic factors, and few comparative studies have addressed if the swarming behavior of *S. gregaria* is actually the same as that of other species (Sword *et al.*, 2000). There is much confusion about the relationship between swarming behavior and density-dependent phase polymorphism. Here I define the swarming behavior as density-dependent morphological, physiological, and behavioral changes found in locusts, leading to a massive multiplication in population and to migration. Density-dependent polymorphism is rather a broad phenomenon found in other Orthopteran species as well as some Lepidopteran species, but the swarming behavior is very specific to locusts (Applebaum and Heifetz, 1999).

Locusts respond to the density behaviorally, morphologically, and physiologically, often resulting as an extremely gregarious population (Uvarov, 1966, 1977). This extreme form of the phenotypic plasticity is commonly referred to as the phase polymorphism. There are three possible phases within a population: solitary, gregarious, and transient phase (Uvarov, 1966). When locusts occur individually without much interaction with other members of species, there are said to be in a solitary phase. When the density increases, locusts tend to congregate and move as a cohesive group. This state is called a gregarious phase. The transient phase describes the state between solitary and gregarious phases. Thus the phase is not a discrete term, but rather a plastic and continuous representation of the locust’s life. The phase polymorphism is density-dependent, but
other environmental factors (temperature, rainfall) as well as genetic factors are involved (Wedekind-Hirschberger, 1999). Solitary locusts behave much like the ordinary grasshoppers and do not cause much harm to the agriculture. The differences between solitary and gregarious phases are summarized in Table 3.1 (Uvarov, 1966, 1977). The most damage is done when locusts become gregarious, that is when they change in color, behavior, and shape to make them capable of a long flight.

<table>
<thead>
<tr>
<th></th>
<th>Solitary Phase</th>
<th>Gregarious Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohesive day-flying (adult)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Marching band (hopper)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Color (hopper)</td>
<td>Green</td>
<td>Dark (Black and Yellow)</td>
</tr>
<tr>
<td>Morphology</td>
<td>Narrow pronotum and sternum</td>
<td>Broader pronotum and sternum; longer wings</td>
</tr>
<tr>
<td>Number of eggs per pod</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Frequency of egg laying</td>
<td>Three times</td>
<td>Twice</td>
</tr>
<tr>
<td>Number of instars</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3.1: Differences between solitary and gregarious phase in locusts

However, morphology alone cannot define the status of a locust because in nature there are gregariously-behaving solitarily-looking locusts and vice versa. In general, when the locusts are experiencing a phase change, the behavior responds first and morphology and physiology change later. These changes are reversible (Uvarov, 1966). Yet, there is no definition of an all-encompassing "absolute" phase state because the
phase characteristics and the amplitude of their changes depend also on species, strain within the species, sex and instar (Pener and Yerushalmi, 1998).

There are several factors influencing the aggregation of *Schistocerca*. Visual, olfactory, and tactile stimuli affect the behavioral change, but the tactile stimulus seems to play an important role in the behavioral change (Roessingh *et al.*, 1998; Hägele and Simpson, 2000). In fact, Simpson *et al.* (2001) showed that the gregarious phase of *S. gregaria* is evoked by touching the hindleg. Simpson *et al.* (1999) presented the comprehensive behavioral assay of *Schistocerca gregaria* leading to the behavioral phase change. Also, there is a maternal effect in this process, and in fact McCaffery *et al.* (1998) found that the color changes probably depend on the maternal material present in the egg pod, which was determined to be a small (3kDa) hydrophilic factor. Several *Schistocerca* species exhibit all three changes (behavior, morphology and physiology) according to the biotic and abiotic pressure. In some cases, the species may exhibit the phase polymorphism, but not form a swarm. For example, *S. lineata*, a sedentary species, also displays certain density-dependent phase characteristics (Sword and Dopman, 1999).

How the swarming behavior and the phase polymorphism evolved is still not clearly understood. Orthopterists generally agree that the swarming behavior is a convergence because it is found in many phylogenetically distant groups. In *Schistocerca*, the *americana* complex contains all four swarming species, and it is tempting to hypothesize that the swarming behavior has evolved once in that clade. The phase polymorphism, although closely associated with the swarming behavior, is a separate event. Sword and Dopman (1999) and Sword *et al.* (2000) studied the density-dependent aposematic coloration in *S. gregaria* and *S. lineata*. He showed that the
gregarious nymphs actively prefer toxic plants whereas the adults are more polyphagous. In a behavioral essay using a lizard as a predator, Sword (1999) demonstrated that the predator quickly learns the aposematic coloration associated with unpalatability and avoids the subsequent feeding. Given that the similar phenomenon is found in the swarming species as well as the sedentary species, it is possible to hypothesize that the common ancestor of two groups had the ability to change colors depending on density and host use. Of course, it is difficult to rule out the possibility of convergence, but Schistocerca is no doubt a fascinating group with many questions to be answered.
3.4. CLADISTIC ANALYSIS

3.4.1. Taxa

Ten North American Schistocerca species including 5 species in the alutacea complex *sensu* Dirsh (1974) and *S. gregaria* were included in the analysis. North American species include *Schistocerca alutacea, S. lineata, S. rubiginosa, S. albolineata, S. shoshone, S. obscura, S. ceratiola, S. damnifica, S. nitens,* and *S. americana.* *Austracris guttulosa, Acanthacris ruficornis, Anacridium aegyptium, Cyrtacanthacris aeruginosa,* and *Halmenus robustus* were used as cyrtacanthacridine outgroups.

Museum specimens from various localities were studied, and at least 10 male genitalia were dissected from each *Schistocerca* species to study individual variations.

3.4.2. Characters

Characters are selected based on morphological studies of Dirsh (1974) and Hubbell (1960). Novel characters from mouthparts, pronotum, and ovipositors were used as well. For better visualization of characters, different views of male genitalia were drawn with the help of camera lucida. Forty-one characters were used, 13 from the male genitalia and 28 from the external morphology. Male genitalia were dissected from relaxed specimens and the muscles dissolved in 10% KOH solution. Endophalli were dissected from the phallic complex to study internal characters. Dissected genitalia were preserved in glycerin. Appendix B shows the characters and character states used in the analysis.
3.4.3. Character coding and Analysis

Parsimony analysis was carried out in this study. All character states were binary and non-additive. *Anacridium aegyptium* was used to root the tree. Missing character was coded as ?. The data matrix was created in WinClada (ver. 0.9.00m24(Beta)) and the cladistic analysis was carried out using NONA (Version 2.0). Search parameters include rs0; mult*50; max*. Appendix A shows the data matrix for the present analysis. A strict consensus was performed from multiple most parsimonious trees (MPTs).

3.4.4. Results

Four most parsimonious trees were obtained. When a strict consensus was performed, three terminal nodes were collapsed (L=101; Ci=40; Ri=61). The genus *Schistocerca* is a monophyletic group (Fig. 3.1., arrow). North American sedentary *Schistocerca* species formed a monophyletic group. Within this clade, the *alutacea* complex *sensu* Dirsh formed a paraphyly because *S. obscura*, which Dirsh (1974) treated as a separate species, was a sister taxon to *S. albolineata* (Fig. 3.1). *Schistocerca nitens* and two species from the *americana* complex formed a monophyletic group, within which *S. americana* and *S. gregaria* are sisters. *Halmenus robustus* was a sister taxon to *Schistocerca*. 
Figure 3.1: The phylogeny of North American Schistocerca. A strict consensus of four MPTs. Solid square indicates a synapomorphy, and the white square indicates a homoplasy. Bold line indicates the alutacea complex sensu Dirsh.
3.5. DISCUSSION

3.5.1. Taxonomic implication

Dirsh (1974) synonymized six species as subspecies of *Schistocerca alutacea* based on the morphometrics. He reasoned that because morphometric characters overlap among species, these species are merely different ecological forms or geoclines. Of the species under the *alutacea* complex, five species, *S. alutacea*, *S. rubigiosa*, *S. lineata*, *S. shoshone*, and *S. albolineata*, occur in the U.S., and *S. insignis* occurs in Mexico. *Schistocerca insignis* is not included in the present analysis because I was unable to obtain the specimens, but based on the fact that Dirsh only examined two males and six females, it is possible that the species are rarely collected. Because Dirsh did not use any biological information when defining a species, and because he treated species in the *americana* complex as subspecies while they are valid species (Harvey, 1981), I treated his subspecies separately in the analysis.

The present phylogeny is revealing in several aspects. The strict consensus tree (Fig. 3.1) shows that the *alutacea* complex *sensu* Dirsh is paraphyletic. It suggests that the previously separate species, *S. obscura*, is a sister to *S. albolineata*. The reason Dirsh did not include *S. obscura* in the *alutacea* complex is not clear despite the fact that *S. obscura* and *S. albolineata* are externally very similar. He included *S. albolineata* in the complex because he claimed to have found the intermediate form between *S. lineata* and *S. albolineata* (Dirsh, 1974: 211). However, this intermediate form is in fact an aposematic morph of *S. lineata* as studied by Sword and Dopman (1999). The strict consensus failed to produce a resolved relationship among the closely related species of
North American *Schistocerca* (Fig. 3.1), but because of the placement of *S. obscura*, the species concept of *S. alutacea sensu* Dirsh is effectively refuted. Although a revision with more robust data is necessary, I argue that the subspecies of *S. alutacea* are indeed valid species. Moreover, the name “*alutacea complex*” needs to be reconsidered. This name has been used for last 40 years since Hubbell’s (1960) partial revision, and it implies that *S. alutacea* is somehow a representative of all the other species. While it is true that there are several closely related species to *S. alutacea*, as shown by the present analysis (Fig. 3.1), the way Dirsh used it is incorrect due to the paraphyly. Because of the traditional value, the “*alutacea complex*” may be used, but it means nothing more than the close affinities among species.

To be more technical, however, *Schistocerca ceratiola* has a very close affinity to the previous “*alutacea complex*.” The monophyletic group that includes from *S. ceratiola* to *S. albolineata* is strongly supported by having the long antennae (character 1), a pair of tubercle on male supra-anal plate (character 2, which *S. obscura* and *S. albolineata* lost) and the bilobate male cerci (character 4). *S. damnifica* is basal to all the other sedentary grasshoppers. Because North American sedentary *Schistocerca* is monophyletic, it is possible to hypothesize that the common ancestor of this lineage colonized the North America and diversified.

North American *Schistocerca nitens* is a sister to the *americana complex*. This species potentially includes several more South American species, but an in-depth taxonomic study is necessary to confirm this idea. As Harvey (1981) proposed, *S. americana* and *S. gregaria* are closely related. Although more taxa need to be included in the analysis to understand the phylogenetic relationships among the species in the
*americana* complex, this analysis suggests that *S. gregaria* is a sister to the New World species.

### 3.5.2. Biogeographical implication

The present analysis provides a new insight into understanding the current distribution of *Schistocerca*. *Schistocerca gregaria* is the only Old World representative of the genus, and it is a sister to a New World species, *S. americana* (Fig. 3.1). Dirsh's (1974) notion of the *americana* complex being the most primitive in the genus is not supported because the clade is nested deep in the cladogram. However, because the present analysis did not include other species in the *americana* complex, the phylogenetic relationship among those species is unknown.

The Old World origin hypothesis assumes that the *Schistocerca gregaria*-like ancestor colonized the New World while the remaining individuals remained unchanged in the Old World. If this is the case, the resulting phylogeny should have *S. gregaria* as the basal species to all the other New World species (Fig. 3.2A). However, *S. gregaria* is in fact a sister to *S. americana*, which makes the Old World scenario unlikely. The New World origin hypothesis is more plausible based on the phylogeny because it only requires single eastward colonization by *S. gregaria* (Fig. 3.2B). The analysis enables to hypothesize the possible diversification scenario. The ancestor *Schistocerca* invaded the New World perhaps by crossing the Atlantic. The invasive flight started from West Africa to the eastern Brazil. From then, the diversification happened rapidly both north and south directions by utilizing unoccupied niches. The Pleistocene glaciations prevented the diversification of North American species, and therefore, the sedentary
Schistocerca species feeding on the herbaceous plants diversified in last one million years. One of the long winged species belonging to the americana complex flew over the Atlantic to recolonize Africa. Surviving members of the swarm landed in West Africa, and because these individuals had the enormous flying capacity, they were able to survive and reproduce. Due to the records about the disastrous locusts in the ancient literatures, people tend to think that the desert locusts are very “ancient,” but comparing the history of humans to the history of grasshoppers, the desert locust has possibly relatively very recent in origin. Unfortunately, however, the phylogeny does not provide a definite answer to the biogeographical question because the analysis is based on the extant taxa and the current distribution. It does not take account into potentially extinct taxa that might have been present in the Old World nor does it consider the biotic factors that contributed to the diversification of the genus. Altogether, the biogeographical distribution of Schistocerca needs more rigorous attention.

One of the most revealing aspects about the present analysis is the age of Schistocerca. Galapagos genus Halmenus is a sister to Schistocerca (Fig. 3.1), which indicates that the diversification of two genera is as recent as the age of the Galapagos Islands. There are two Schistocerca species (S. melanocera and S. literosa) present in the Galapagos Islands, but these seem to be the result of a separate, more recent invasion because these Schistocerca species are macropterous and highly mobile. There are only two known genera of Cyrtacanthacridinae in the New World, while there are over 30 genera in the Old World (Otte and Naskrecki, 1997). Although the concept of center of origin is difficult to test, orthopterists generally agree that Cyrtacanthacridinae originated from Africa (Dirsh, 1974; Amedegnato, 1993). Therefore, it is likely that the New World
cyrtacanthacridine fauna is a direct result of westward colonization. Because the present phylogeny places *Halmenus* as a sister to *Schistocerca*, it is conceivable that the common ancestor of *Halmenus* and *Schistocerca* invaded the New World once by a rare occasion and diversified. There may have been several extinctions between the *Halmenus* clade and *Schistocerca* clade. The genus *Schistocerca* is well defined by the morphology of cerci and subgenital plate, whereas *Halmenus* retain several plesiomorphic characters found in other cyrtacanthacridine genera. Therefore, the diversification of *Schistocerca* is probably the more recent event than the diversification of *Halmenus*. This can suggest that the age of *Schistocerca* is at least two million years old. *Schistocerca* is the most speciose genus in Cyrtacanthacridinae, and achieving the present diversity in such a short period of time is overwhelming.
Figure 3.2: Hypothetical scenarios of *Schistocerca* diversification. A: The Old World origin hypothesis. *S. gregaria*-like ancestor colonized the New World while the remaining individuals populated in the Old World. *Halmenus* is the result of separate colonization. B: The New World origin hypothesis. The common ancestor of *Halmenus* and *Schistocerca* colonized the New World once, and *S. gregaria* re-colonized the Old World.
3.5.3. Evolutionary implication

The present analysis allows hypothesizing the possible scenario of the evolution of
swarming in Schistocerca, but because it only included single swarming species, S.
gregaria, the result has a limited value. There are four swarming species in Schistocerca,
S. piceifrons, S. cancellata, and S. interrita, and S. gregaria, and more comprehensive
analysis including all the swarming species would be necessary. Given that these species
belong to the americana complex (Harvey, 1981), it is certainly possible to hypothesize
that the complex forms a monophyletic group. Moreover, a behavioral character such as
swarming is a composite character, and it needs to be broken down into several small
discrete components. Altogether, the present phylogeny is not the best way to test the
evolution of swarming, but it certainly reveals a new insight into understanding the
evolutionary pattern.

How did swarming behavior evolve in Schistocerca? If the ancestral Schistocerca
invaded the New World, the ability to swarm might have been already present. But, it
seems as though this trait has been lost in the genus because most species in the genus are
sedentary. The swarming behavior might have reappeared in the americana complex.
However, even all the sedentary Schistocerca species are very strong fliers, and this flight
capacity is perhaps a plesiomorphic character. Recent discovery of the swarming S.
interrita, which probably belongs to the americana complex (Dirsh, 1974), indicates that
certain species may have a potential to swarm and will swarm if a favorable condition is
provided. This in turn implies that there are possibly a few more swarming Schistocerca
which have not been exposed to the environmental conditions to swarm. Thus, it is
conceivable to see that swarming behavior is an evolutionary character although the behavior is a complicated response to both biotic and abiotic environments.
### APPENDIX

**Appendix A. Data matrix**

| Taxa                       | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| *Anacridium aegyptium*     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  | 1  | 0  | 1  | 0  | 0  | 1  | 0  | 0  |
| *Austacris guttulosa*      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  |
| *Acanthacris ruficornis*   | 0  | 0  | 0  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 1  | 1  | 0  | 0  | 1  | 0  | 0  | 0  |
| *Cyrtacanthacris aeruginosus* | 0  | 0  | 0  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  |
| *Halmenus robustus*        | 0  | 0  | 0  | 0  | 0  | ?  | 0  | 0  | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 1  | 0  |
| *S. americana*             | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 0  | 1  | 1  |
| *S. gregaria*              | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 1  |
| *S. nitens*                | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  |
| *S. alutacea*              | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 0  |
| *S. rubiginosa*            | 1  | 0  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 1  | 1  | 0  | 1  | 1  |
| *S. lineata*               | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 0  | 0  | 1  |
| *S. shoshone*              | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 0  | 0  | 0  |
| *S. obscura*               | 1  | 1  | 1  | 0  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 1  | 1  | 0  | 1  | 0  | 1  | 1  |
| *S. albolineata*           | 1  | 1  | 1  | 0  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 1  | 0  | 1  | 1  |
| *S. damnifica*             | 0  | 0  | 1  | 0  | 1  | 0  | 1  | 1  | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  |
| *S. ceratiola*             | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 1  |
### Appendix A. Data matrix (cont’d)

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Appendix B. Character description and character states

[1] Antennae length
  0: as long as head+pronotum; 1: much longer
[2] Bilobate male cercus
  0: absent; 1: present
[3] Male cercus narrowing toward apex
  0: yes; 1: no
  0: absent; 1: present
[5] Male subgenital place narrowing toward apex
  0: no; 1: yes
[6] Round notch in male subgenital plate
  0: absent; 1: present
[7] Tegmina length
  0: extending much beyond the tip of abdomen; 1: slightly extending beyond the tip of abdomen
[8] Tegmina color
  0: transparent; 1: semi-transparent
[9] Hindwing color
  0: absent; 1: present
[10] Elevated median carina
   0: absent; 1: present
   0: absent; 1: present
[12] Pronotum: Lateral side of prozona
   0: smooth; 1: wrinkled
[13] Straight prosternal process
   0: absent; 1: present
[14] Prosternal process narrowing toward apex
   0: no; 1: yes
[15] Deeply cut pronotum sulci
   0: absent; 1: present
[16] Thickened frontal femur
   0: absent; 1: present
[17] Head: base of frontal ridge projection
   0: absent; 1: present
[18] Parallel frontal ridge
   0: absent; 1: present
[19] Mouthparts: Right mandible incisor lobe
   0: not elongated; 1: elongated
[20] Mouthparts: Right mandible molar dentes
   0: dull; 1: pointed
[21] Mouthparts: Left mandible incisor lobe
   0: not elongated; 1: elongated
[22] Mouthparts: Maxillary basal projection
   0: absent; 1: present

[23] Epiphallus: Anterior projection: lamelliform
   0: absent; 1: present

[24] Epiphallus: Bridge length longer than width of anterior projection
   0: no; 1: yes

[25] Cingulum: Bulbous rami of cingulum
   0: absent; 1: present

[26] Ectophallic sclerite: Constricted median projection
   0: absent; 1: present

[27] Endophallus: Arch of cingulum curved proximally
   0: no; 1: yes

[28] Endophallus: Valve of cingulum curved downward
   0: no; 1: yes

[29] Endophallus: Broad basal valve of penis
   0: absent; 1: present

[30] Endophallus: Constricted gonopore process
   0: absent; 1: present

[31] Endophallus: Apical valve of penis narrowing toward apex
   0: no; 1: yes

[32] Cingulum: Sclerotized zygoma
   0: absent; 1: present

[33] Epiphallus: Width of epiphallus
   0: long; 1: short

[34] Endophallus: Short apical valve of penis
   0: absent; 1: present

[35] Ectophallic sclerite: Length
   0: long; 1: short

[36] Bilobate male subgenital plate
   0: absent; 1: present

[37] Quadrate male cercus
   0: absent; 1: present

[38] Head: Frontal ridge reaching clypeus
   0: no; 1: yes

[39] Ovipositor: Lower basivalvular sclerite tubercle
   0: absent; 1: present

[40] Ovipositor: Slender dorsal/ventral valves
   0: absent; 1: present

[41] Ovipositor: Proximal basivalvular sclerite slender
   0: no; 1: yes
BIBLIOGRAPHY


