POST-ADULT EMERGENCE DEVELOPMENT OF GENITALIC STRUCTURES IN *SCHISTOCERCA* STÅL AND *LOCUSTA* L. (ORTHOPTERA: ACRIDIDAE)

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Abstract.—The male genitalia and female ovipositor in *Schistocerca americana* (Drury) (Orthoptera: Acrididae: Cyrtacanthacridinae) continue to develop after adult emergence. The internal skeleton comprising the phallic complex is affected by cuticle deposition, resulting in qualitative shape changes during sexual maturation. Lateral apodemes of the ovipositor also grow in length and width during sexual maturation. Similar developmental patterns are found in the male genitalia of *S. gregaria* (Forskål) and *Locusta migratoria* (Linnaeus), suggesting the possibility that post-adult emergence genitalic development may be widespread within Acridoidea. Newly emerged individuals may be functionally incapable of copulation because the necessary structures have not been fully matured. Taxonomic use of the genitalic structures is discussed in light of the present finding.

Key Words: post-adult emergence, cuticle development, genitalia, grasshopper, taxonomy

The final molt in insect development results in the adult instar responsible for the reproduction. While there are some groups that are capable of copulating immediately after emergence (Ridley 1989), many insects have a period of sexual maturation with the adult instar. The post-emergence maturation period of *Schistocerca* Stål (Orthoptera: Acrididae) is about 30 days, which is very long compared to that of other insects (Norris 1954, Ridley 1988, Weis-Fogh 1952). Historically, the gonads have been considered the only developing structures responsible for delayed sexual maturation (Norris 1952, 1954, 1957). For example, Norris (1954) documented the growth of the egg-rudiments in females and underdeveloped receptaculum seminis in males of sexually immature locusts. However, physiological studies revealed that developmental changes at cuticular, muscular, and ultrastructural levels occur throughout the teneral period (Neville 1963a, Weis-Fogh 1952, Viscuso et al. 1985). Significant maturation in the adult stage is known from Orthoptera, Dermaptera, Odonata, Hemiptera, Homoptera, Hymenoptera, and Diptera (Table 1). Structures that undergo this process include muscles, corpora allata, epithelium, and both internal and external skeletal elements. The period of development can be as long as 35 days (Table 1).

The developmental changes documented until now are, however, quantitative changes that deal with the increase in thickness or volume. I demonstrate below that the genitalia undergo a qualitative change in shape during the entire period of the sexual maturation in *Schistocerca americana* (Drury). Also, I explore the taxonomic implications of this finding because the genitalic characters have been used extensively in descriptive taxonomy (Dirsh 1973, Eberhard 1985).

Table 1. Post-adult emergence development of different structures and their processes and timing along with orders known to have the phenomena.

Structure	Process	Post-emergence Timing	Order Found	Author
Exoskeleton	Chitin layer deposition from epidermis	Until sexual maturity	Orthoptera, Dermaptera, Odonata, Hemiptera, Homoptera, Hymenoptera	Neville (1963b), Neville (1983)
Thoracic apodemes	Resilin deposition	One week	Orthoptera	Neville (1963a)
Genital apodemes	Resilin deposition	12 days	Diptera	Schlein (1972)
Thoracic muscles	Increase in volume of mitochondria and contractile protein	Until sexual maturity	Diptera, Orthoptera	Bursell (1961), Finlayson (1975), Weis-Fogh (1952)
Corpora allata	Development of nuclei and allatum-cell cytoplasm	Until sexual maturity	Orthoptera	Odhiambo (1966)
Ejaculatory duct epithelium	Release of Golgi vesicles and lysis of cells	Until sexual maturity	Orthoptera	Viscuso et al. (1985)
Male phallus	Resilin deposition	21–35 days	Orthoptera	This study
Lateral apodemes of female ovipositor	Resilin deposition	20–33 days	Orthoptera	This study

MATERIALS AND METHODS

From laboratory colonies, the last nymphal instars of both male and female *Schistocerca americana* were collected and transferred to a clean cage. When the nymphs molted to adults, they were placed in separate tubular cages, approximately 7 cm in diameter and 30 cm in height, with a steel 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 if an individual died during the course of experiment, it was immediately removed. Locusts were daily fed fresh Romaine lettuce and wheat bran, and maintained at 30°C, 12:12 Light:Dark cycle. One cage of insects was killed each day to sample development across 35 days of post-adult maturation. Insects were killed each day by freezing at −20°C for 30 minutes to collect developmental data. After the genitalia were dissected, specimens were stored in 95% ethanol. Ninety-five male genitalia and thirty-five ovipositors were examined in this study. Fifteen *Schistocerca gregaria* (Forskål) and fifteen *Locusta migratoria* (Linnaeus) (5 freshly molted, 5 2-week-old, and 5 sexually mature for each species) were also dissected. Sexual maturity for these species was determined by both time after hatching and yellow coloration (Stephen Roberts, personal communication). All three species were reared under crowded condition to avoid any potential density-dependent effects known to exist in locusts.

Male genitalia were extruded by inserting a probe under the supra-anal plate, using the technique described by Hubbell (1932). When the phallic complex was exposed, the surrounding membranes were removed. Ovipositors were dissected by making a slit at the distal part of abdomen. Brief descriptions of color and morphology were recorded daily. Genitalia were placed in 10% KOH solution for several hours to dissolve muscles. Cleared genitalia were placed in a vial filled with glycerin, and each genitalic specimen was given an identification num-
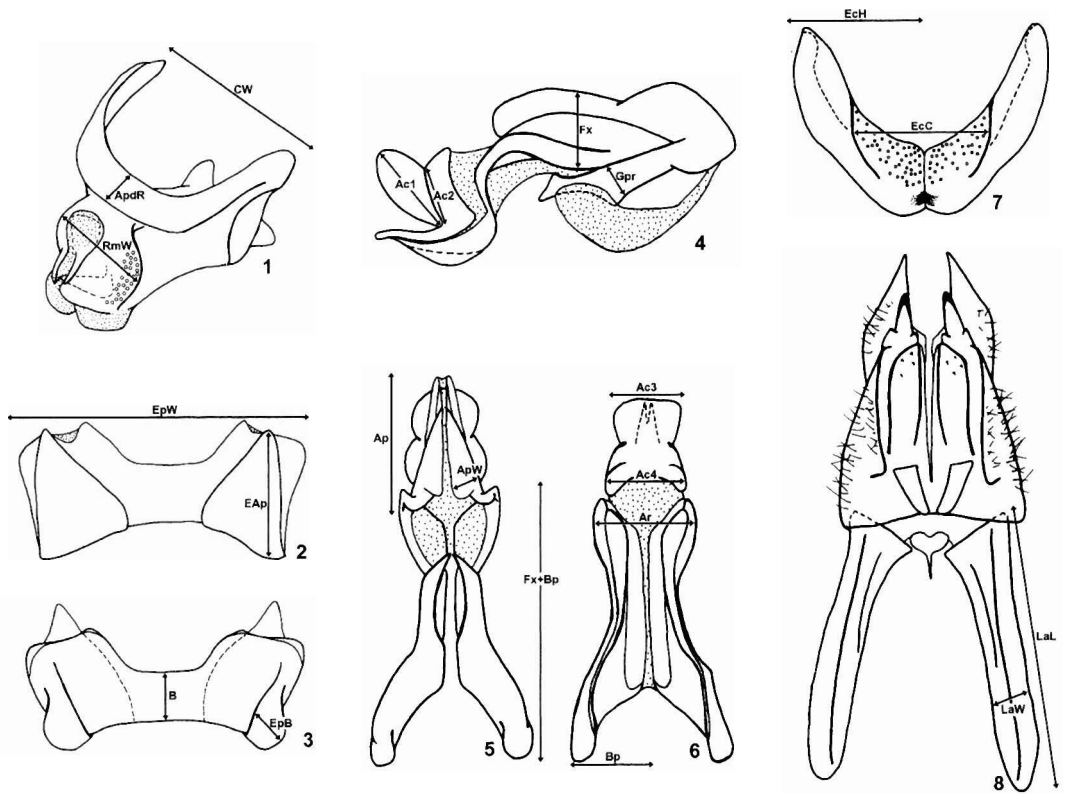
Figs. 1–8.   Dimensions of the *Schistocerca americana* phallic complex and ovipositor measured in the study. 1, Cingulum (RmW: width of rami; ApdR: width of apodeme ridge; CW: width of cingulum). 2, Dorsal view of epiphallus (EpW: horizontal width; EAp: dorso-ventral length of anterior projection). 3, Ventral view of epiphallus (B: dorso-ventral width of bridge; EpB: width of bulbous base). 4, Lateral view of endophallus (Ac1 and Ac2: length of arch of cingulum; Fx: dorso-ventral width of flexure; Gpr: width of gonopore process). 5, Ventral view of endophallus (Ap: length of apical valve; ApW: width of apical valve; Fx+Bp: longitudinal length of flexure + basal valve). 6, Dorsal view of endophallus (Ac3 and Ac4: width of arch of cingulum; Ar: width of arms to apical valve; Bp: lateral width of half of basal valve). 7, Ectophallic sclerite (EcH: horizontal width of half; EcC: horizontal width of midprojection). 8, Ovipositor (LaL: longitudinal length of lateral apodeme; LaW: width of lateral apodeme).

ber. Twenty different dimensions of male genitalia and length and width of the lateral apodemes of ovipositors were measured using ocular micrometer attached to a dissecting scope (Figs. 1–8). Because the phallic complex is comprised of several parts, the dimensions of the cingulum were measured first, and the endophallus was dissected afterwards. Terminology used here followed that of Dirsh (1956). All measurements were logarithmically transformed to normalize variance and plotted against the days after emergence using MINITAB.

RESULTS

Developmental sequence of the genitalia.—Differences between the genitalia of each specimen and those of a sexually mature male were documented in order to describe the developmental stage of a certain age. Although most individuals followed similar patterns, some seemed to develop more slowly than others. External ovipositors did not change, but internal lateral apodemes grew as muscles differentiated through time. However, it was not possible to document the cuticle deposition sequence

of these lateral apodemes, because they were still covered with muscles. Day 1 indicates the first day after the emergence.

Day 0–1: No structure was sclerotized and the color of phallic complex was opaque and white. Epiphallus was fully formed, but the cuticle was very thin and white in color. Cingulum, ectophallic sclerite, and endophallus were not fully formed and appeared to be very fragile. The arch of cingulum in endophallus was absent.

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

Day 3: The lateral parts of rami began to be filled with cuticle and were brown.

Day 4: All structures were more robust due to cuticle deposition. In one male, the distal projection of the arch of cingulum had started to develop. It was very thin and cylindrical and appeared to originate from below both zygoma and the base of arch of cingulum.

Day 5: The structure found in one male on day 4 appeared in all males.

Day 6–7: More cuticle deposition was in process, but not much different from the day 5. The amount of cuticle deposition varied as if certain individuals had developed faster than others.

Day 8–9: The arch of cingulum became gradually thicker and larger compared to the same structure of earlier days.

Day 10: Cuticles became more rigid, and 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. The arch of cingulum was getting thicker, but still a cylindrical shape.

Day 12–13: The arch of cingulum became gradually thicker than the same structure of earlier days, and other parts became more robust.

Day 14–16: Tip of anterior projection of epiphallus became darker, and the hourglass shaped portion below zygoma became even smaller.

Day 17–20: Morphology of phallus was similar to that of a fully mature male. The arch of cingulum became bulbous. External coloration of locusts was brown still indicating sexual immaturity.

Day 21: Some locusts turned bright yellow, indicating sexual maturity.

Day 22–30: Most locusts turned yellow and all of these had a fully mature phallus. During this period, occasional copulation and oviposition were observed. On day 30, there were still males with brown coloration, which did not have a fully mature phallus.

Day 31–35: All the locusts were bright yellow and the phallic complex was highly sclerotized and mature.

Rate of development.—Cuticle deposition occurred throughout the parts of phallic complex during sexual maturation, resulting in an increase in thickness (Figs. 9–12). Of twenty dimensions measured, most dimensions did not result in significant changes during development. For some dimensions, however, cuticle deposition was significant enough to change the shape of the structure. In cingulum, overall size remained constant throughout maturation period (CW in Fig. 9), but the apodemes of cingulum (ApdR in Fig. 9) became thicker and wider as the insect matured (Fig. 13). Likewise, overall size of epiphallus remained constant (EpW in Fig. 10), but there was a gradual increase in size of the bulbous base of epiphallus (EpB in Fig. 10, Fig. 14). Resulting developmental patterns of dramatically changing dimensions in both cingulum and epiphallus may be represented as a curve where growth approaches an asymptote. Endophallus showed the most qualitative changes, resulting in dramatic morphological changes. Laterally, the distal projection of the arch of cingulum, which was absent for the first three days after emergence, sud-
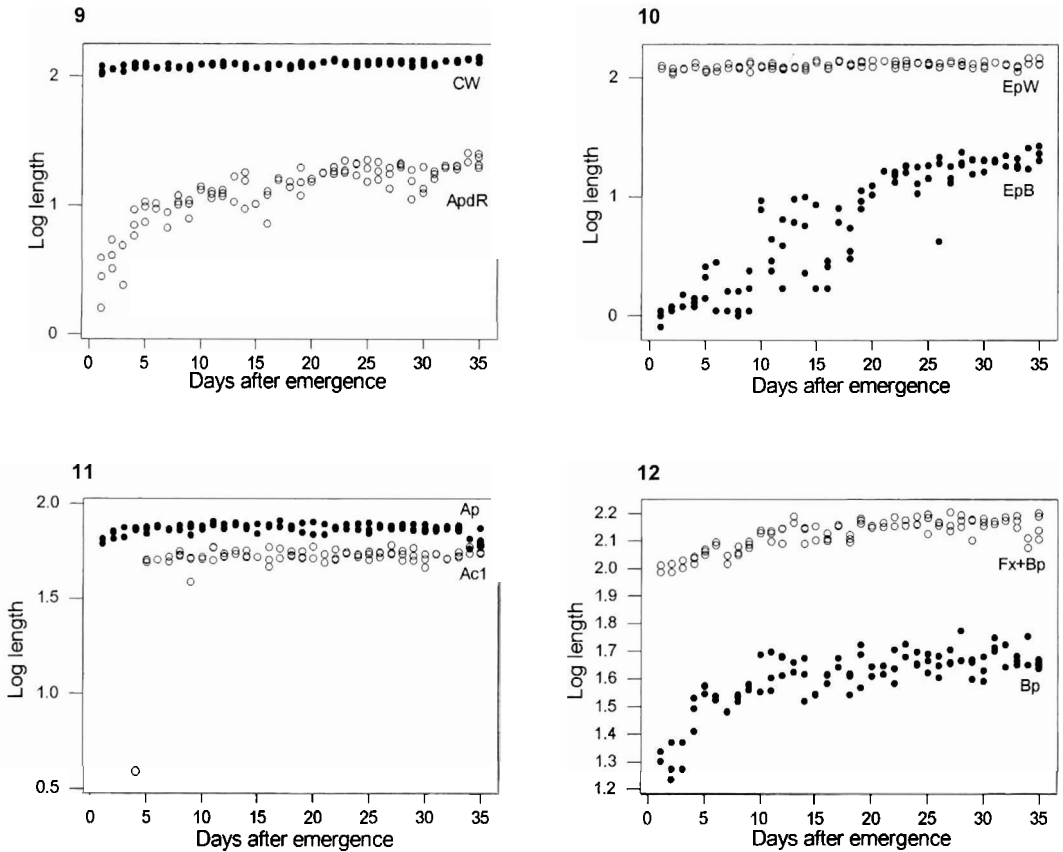
Figs. 9–12. Logarithmically transformed measurements were plotted against days after adult-emergence to show the developmental patterns of each measured structure. In each figure, dimensions from the same genitalic structure were plotted. 9, In cingulum, entire width of cingulum (CW) remains constant whereas width of apodeme ridge (ApdR) changes. ApdR starts to mature around 14 days after emergence. 10, In epiphallus, width (EpW) remains constant whereas width of bulbous base (EpB) gradually develops over time and starts to mature around 26 days after emergence. 11, In endophallus, length of apical valve (Ap) remains constant, whereas arch of cingulum (Ac1) suddenly appears at day 4 after emergence. Arch of cingulum does not grow lengthwise after it forms, but it gradually thickens until 20 days after emergence (not shown). 12, In endophallus, both width of basal valve (Bp) and length of flexure + basal valve (Fx+Bp) gradually increase and start to mature around 10 days after emergence.

denly began developing four days after the emergence (Ac1 in Fig. 11, Fig. 15). It started as a thin cylindrical structure and became bulbous (Fig. 15). The only non-changing part was the length of apical valve of the penis (Ap in Fig. 11, Figs. 15, 16). Ventrally, the most significant morphological changes can be found in gonopore process and basal valve (Bp in Fig. 12, Fig. 16). These structures were not differentiated immediately after the adult emergence, but rapidly developed until ten days after emer-

gence (Fig. 12). Overall, the size of endophallus increased during maturation mainly due to the growth of flexure and basal valve (Figs. 12, 15, 16). The distal part of endophallus remained relatively constant, and the proximal part elongated through cuticle deposition. The ejaculatory sac was present immediately after the emergence, but the cellular maturity was not measured (Figs. 15, 16). Ectophallic sclerite did not change in size except for the increase in cuticle thickness. In females, the lateral apodemes

of ovipositor continued to develop in length and width after emergence (Fig. 17). The external valves did not change in size except that the muscle mass inside the valves increased.

Post-emergence development of male genitalia in other species.—The phallic complexes of *Schistocerca americana* and *S. gregaria* are very similar, but the phallic complex of *L. migratoria* differ morphologically. The size of the whole insect was similar between *Schistocerca* and *Locusta,* but *L. migratoria* has a phallic complex three times as large as that of *Schistocerca.* The entire phallic complex of *L. migratoria* is highly sclerotized immediately after emergence, indicated by the brown color of cuticle.

Figures 18a and 19a show the development of the cingulum in *S. gregaria* and *L. migratoria,* based on specimens that were freshly molted, 2 weeks after molting, and 35 days after molting. In both cases, the apodemes of cingulum increased in width and length. The distal portion of cingulum also remained relatively unchanged, whereas the increase in cuticle deposition of the proximal portion was dramatic. In both *Schistocerca* species, width of the bulbous base of epiphallus in width through time. In *L. migratoria,* no structure increased in width, but thickness of cuticle increased through time. In both *Schistocerca* species, the arch of the cingulum was absent immediately after emergence and progressed from a cylindrical to a bulbous shape (Figs. 15, 18b). Both basal valve of penis and gonopore process 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. 19b).

DISCUSSION

Genitalia as apodemes.—This study suggests that adult grasshoppers are functionally incapable of mating during sexual mat-uration. Sexual maturity is here defined as a period when both males and females can functionally copulate and produce viable offspring. Each component of the phallic complex experiences a structural change that seems to be closely associated with the muscles responsible for movement during copulation. Growth of the phallic apodemes and lateral apodemes of the ovipositor in *Schistocerca americana* is accompanied by an increase in muscle mass. Elastic nature and cuticle deposition patterns all indicate that the male genitalia and the lateral apodemes of ovipositor probably contain resilin (Keffer and Babcock 1998, Neville 1963b, Tatham and Shewry 2002). In freshly emerged specimens, cuticle deposition has yet to occur and muscle cells have not differentiated. Timing of the complete development of genitalia and timing of sexual maturity coincide, suggesting that mature genitalia are the functional necessity of the copulation. In the colony where both sexually immature and mature specimens were reared together, copulation was observed only between bright yellow individuals, which always had fully mature genitalic structures. Until now, delayed mating has been explained only by the developmental time of soft reproductive structures such as epithelium, ejaculatory duct, and ovaries (Norris 1954, 1957). While these structures are crucial for reproduction, skeletally mature structures that are responsible for copulation, ejaculation, and oviposition are essential for organisms to function properly. This study thus proposes a new proximate causal reason for the delayed mating.

Post-emergence genitalic development is widespread.—Post-adult emergence development of genitalic structures appears to be widespread, at least in Acrididae. *Schistocerca* and *Locusta* belong to two subfamilies, Cyrtacanthacridinae and Oedipodinae, respectively, and their genitalic morphologies differ greatly. However, both genera follow similar developmental patterns in terms of genitalia. For example, the epiphallus, ectophallic sclerite, and cingulum
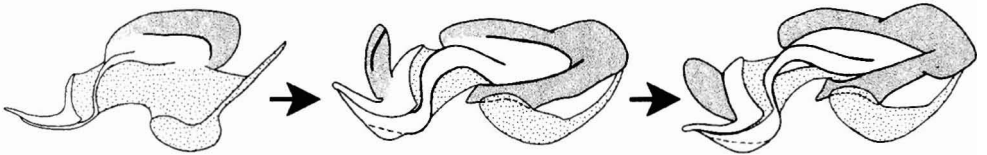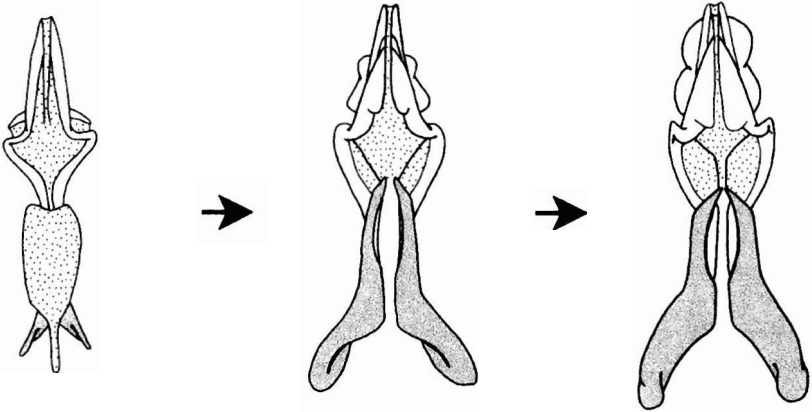
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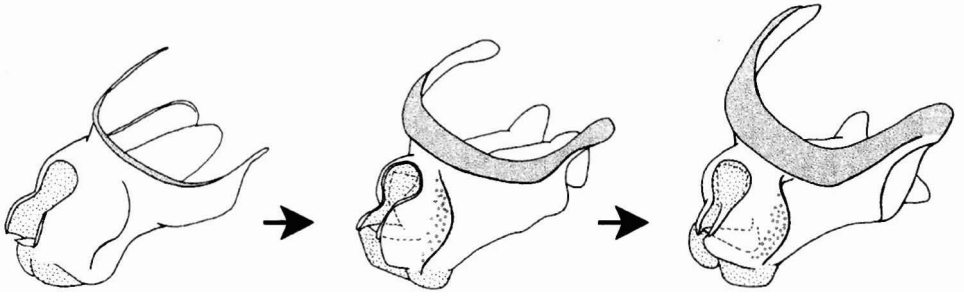
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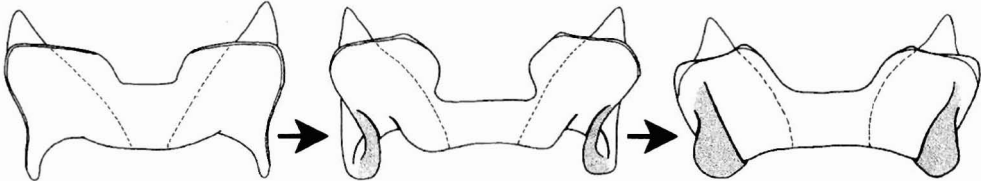
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Emergence   Maturity

Figs. 13–16.   Graphical representation of qualitative developmental changes in *Schistocerca americana* phallic complex (left: immediately after emergence; middle: two weeks after emergence; right: sexual maturity). 13, Cingulum: overall size remains constant, but apodemes of cingulum becomes thicker and wider. Cuticles on rami become thicker and more evident. 14, Epiphallus: overall size remains constant, but base gradually becomes bulbous and sclerotized. 15, Endophallus (lateral): arch of cingulum and gonopore process are not present in freshly emerged individual, but they appear in later developmental stages. Arch of cingulum progresses from a cylindrical to a bulbous shape. 16, Endophallus (ventral): basal valve gradually becomes longer and wider.

17

Emergence   **Maturity**
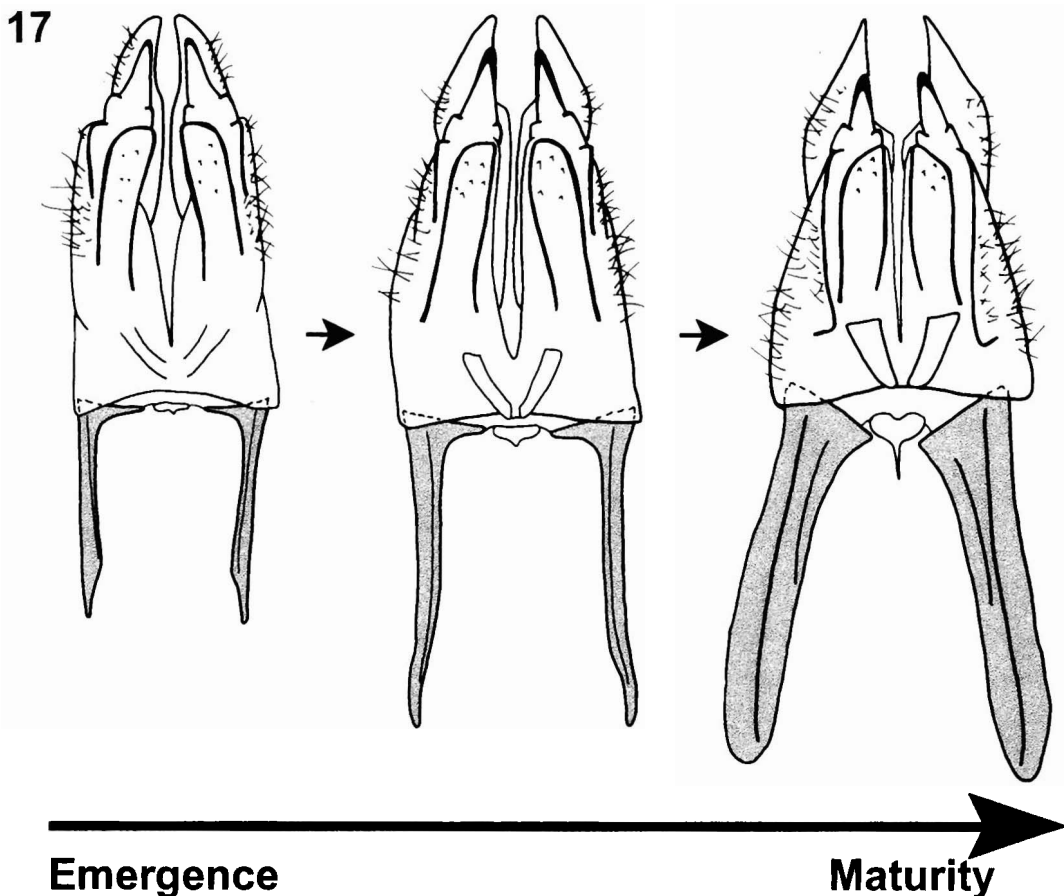
Fig. 17.   Graphical representation of qualitative developmental changes in *Schistocerca americana* ovipositor (left: immediately after emergence; middle: two weeks after emergence; right: sexual maturity). External ovipositor does not change, but lateral apodemes grow longer and wider.

remain relatively constant in size, and the proximal portion of endophallus experiences dramatic structural changes.

How widespread is the post-adult emergence genitalic development? Neville (1983) listed the adult insects shown to have daily growth layers in the cuticle, most of which were hemimetabolous. Although he did not specifically study the internal apodemes, he demonstrated that genitalic development could be found in many hemimetabolous insects. Ridley (1989) documented the time of mating after emergence for the most insect orders. Although there are some exceptions, hemimetabolous insects seem to have longer post-emergence maturation periods compared to holometab-

olous insects. Most holometabolous insects mate immediately after emergence (Ridley 1989), perhaps because all the necessary development occurs during the pupal stage. To date, however, there is no study that focuses on the post-adult emergence genitalic development in any insect group. This neglected area of study deserves more attention because the post-adult emergence genitalic development may in fact be very widespread.

Taxonomic use of genitalia.—Historically, the male genitalia have been used in insect taxonomy extensively (Eberhard 1985). In Acridoidea, many species have a distinct male genitalic morphology (Hubbell 1932, 1960; Dirsh 1956, 1973; Cohn and Cantrall
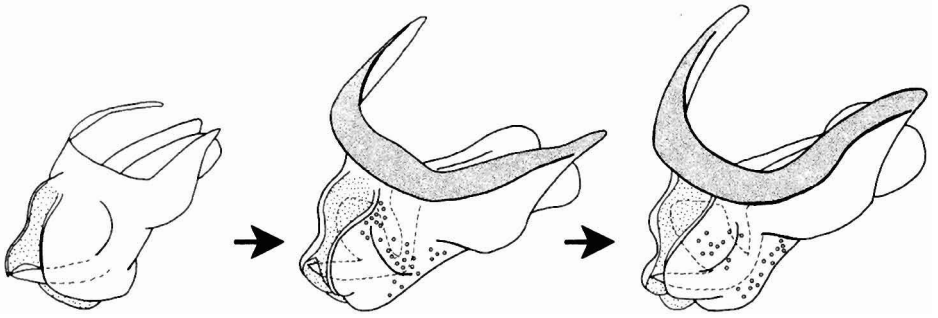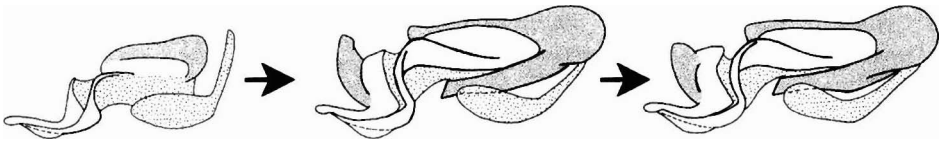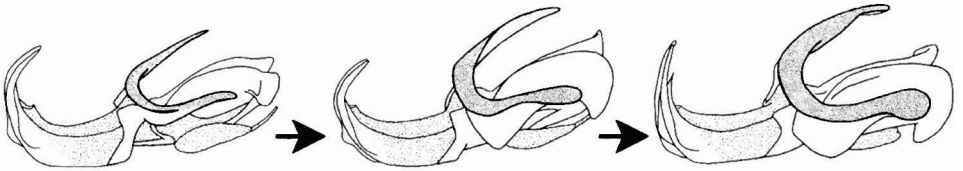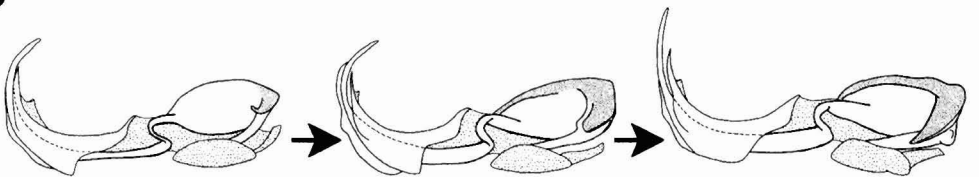
Figs. 18–19. Comparison of post-adult emergence developmental patterns between *Schistocerca gregaria* and *Locusta migratoria* (left: immediately after emergence; middle: two weeks after emergence; right: sexual maturity). 18a, *S. gregaria* cingulum. As in *S. americana*, apodemes of cingulum become thicker and wider. 18b, *S. gregaria* endophallus (lateral). As in *S. americana*, arch of cingulum and gonopore process are not present in freshly emerged individual, but they appear in later developmental stages. 19a, *L. migratoria* cingulum. Freshly emerged individuals have more sclerotized phallus than *Schistocerca*, but likewise apodemes of cingulum gradually develop. 19b, *L. migratoria* endophallus (lateral). Basal valves start as a small structure, but ventrally elongates over time. Arch of cingulum is not found in *Locusta*.

1974). In the North American grasshoppers, *Schistocerca alutacea* (Harris), *S. lineata* Scudder, and *S. rubiginosa* (Harris), male genitalia are the only reliable characters for species differentiation (Hubbell 1960). Despite its significance, however, the variability of the phallic complex within a species is rarely mentioned in the taxonomic literature (Linsley 1939, Shapiro 1978), even though morphological characters generally require a study of large samples. Perhaps, practical issues such as destruction of specimens and laborious preparation prevent taxonomists from examining a large number of specimens.

Developmental aspects need to be considered when using genitalia in taxonomy, especially in Acridoidea. Most taxonomic studies deal with museum specimens in which researchers cannot know the exact age of the specimens. Although there is a method to estimate the age based on the age ring in cuticles (Neville 1963c), it is rarely used. The fact that sexually immature grasshoppers have less developed genitalia than sexually mature ones presents a potential danger in using genitalia in taxonomy. If a species is initially defined using the less developed genitalia, a later taxonomist may interpret the same species to be different based on the fully developed genitalia. In fact, Dirsh (1974: 166) may have defined *Schistocerca braziliensis* based on immature male genitalia. His drawing of the endophallus clearly showed the characteristic of undeveloped arch of cingulum. It is also dangerous to define a species based on the length and width of apodemes because the lateral apodemes of the ovipositor clearly grow during sexual maturation. In Microlepidoptera, Busck (1931) used the length and the sclerotization of apophyses in characterizing families, but there is a possibility that these apodemes can develop after adult emergence (Neville 1983). Though these observations are based on a small number of species, if the phenomenon is widespread, one needs to be cautious about using genitalic characters, because arrested cuticle deposition in museum specimens could be misleading.

LITERATURE CITED

Bursell, E. 1961. Post-teneral development of the thoracic musculature in tsetse flies. Proceedings of the Royal Entomological Society of London (A) 36: 69–74.

Busck, A. 1931. On the female genitalia of the Microlepidoptera and their importance in the classification and determination of these moths. Bulletin of the Brooklyn Entomological Society 26: 199–216.

Cohn, T. J. and I. J. Cantrall. 1974. Variation and speciation in the grasshoppers of the Conalcaeini (Orthoptera: Acrididae: Melanoplinae): The lowland forms of Western Mexico, the genus *Barytettix*. San Diego Society of Natural History, Memoir 6, 131 pp.

Dirsh, V. M. 1956. The phallic complex in Acridoidea (Orthoptera) in relation to taxonomy. Transactions of the Royal Entomological Society of London 108: 223–356.

———. 1973. Genital organs in Acridomorphoidea (Insecta) as taxonomic character. Zeitschrift für Zoologische Systematik und Evolutionsforschung 11: 133–154.

———. 1974. Genus *Schistocerca* (Acridomorpha, Insecta). Dr. W. Junk B.V. Publishers, The Hague, 238 pp.

Eberhard, W. G. 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Massachusetts, 244 pp.

Finlayson, L. H. 1975. Development and degeneration,

pp. 75–149. *In* Usherwood, P. N. R., ed. Insect Muscle. Academic Press, London, 621 pp.

Hubbell, T. H. 1932. A revision of the Puer group of the North American genus *Melanoplus,* with remarks on the taxonomic value of the concealed male genitalia in the Cyrtacanthacridinae (Orthoptera, Acrididae). Miscellaneous Publications, Museum of Zoology, University of Michigan 23: 1–64.

———. 1960. The sibling species of the Alutacea group of the bird-locust genus *Schistocerca* (Orthoptera, Acrididae, Cyrtacanthacridinae). Miscellaneous Publications Museum of Zoology, University of Michigan 116: 1–91.

Keffer, S. L. and S. K. Babcock. 1998. Resilin in waterscorpion male genitalia (Insecta: Heteroptera: Nepidae). American Zoologist 38: 165A.

Linsley, A. W. 1939. Variation of insect genitalia. Annals of the Entomological Society of America 32: 173–176.

Neville, A. C. 1963a. Daily growth layers in locust rubber-like cuticle influenced by an external rhythm. Journal of Insect Physiology 9: 177–186.

———. 1963b. Growth and deposition of resilin and chitin in locust rubber-like cuticle. Journal of Insect Physiology 9: 265–278.

———. 1963c. Daily growth layers for determining the age of grasshopper populations. Oikos 14: 1–8.

———. 1983. Daily cuticular growth layers and the teneral stage in adult insects: A review. Journal of Insect Physiology 29: 211–219.

Norris, M. J. 1952. Reproduction in the desert locust (*Schistocerca gregaria* Forskål) in relation to density and phase. Anti-Locust Bulletin 13: 1–51.

———. 1954. Sexual maturation in the desert locust (*Schistocerca gregaria* Forskål) with special reference to the effects of grouping. Anti-Locust Bulletin 18: 1–44.

———. 1957. Factors affecting the rate of sexual maturation of the desert locust (*Schistocerca gregaria* Forskål) in the laboratory. Anti-Locust Bulletin 28: 1–26.

Odhiambo, T. R. 1966. Ultrastructure of the development of the corpus allatum in the adult male of the desert locust. Journal of Insect Physiology 12: 995–1002.

Ridley, M. 1988. Mating frequency and fecundity in insects. Biological Review 63: 509–549.

———. 1989. The timing and frequency of mating in insects. Animal Behaviour 37: 535–545.

Schlein, Y. 1972. Factors that influence the post-emergence growth in *Sarcophaga falculata.* Journal of Insect Physiology 18: 199–209.

Shapiro, A. M. 1978. The assumption of adaptivity in genital morphology. Journal of Research on the Lepidoptera 17: 68–72.

Tatham, A. S. and P. R. Shewry. 2002. Comparative structures and properties of elastic proteins. Philosophical Transactions of the Royal Society of London (B) 357: 229–234.

Viscuso, R., G. Longo, and L. Sottie. 1985. Ultrastructural modifications in the ejaculatory duct epithelium of *Eyprepocnemis plorans* (Charp.) (Orthoptera: Acrididae) during sexual maturation. International Journal of Insect Morphology and Embryology 14: 163–177.

Weis-Fogh, T. 1952. Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria* Forskål). Philosophical Transactions of the Royal Society of London (B) 237: 1–36.