INTERACTIONS BETWEEN *SCHISTOCERCA GREGARIA* (FORSKÅL) AND *LOCUSTA MIGRATORIA MIGRATORIOIDES* (REICH & FARMAIRE) IN RELATION TO PHASE POLYMORPHISM

BY

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A Thesis

presented to the Department of Crop Science of the

Faculty of Agriculture, University of Ghana, Legon

in fulfilment of the requirements

for the degree of Doctor of Philosophy in

Crop Science (Entomology)

BY

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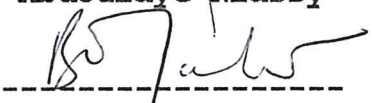
DECLARATION

I hereby declare that the work herein now submitted as a thesis for the Doctor of Philosophy Degree in crop Science (Entomology) is the result of my own investigations and has not been submitted for a similar degree in any other University.

Abdoulaye Niassy



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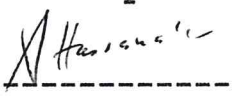

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DEDICATION

To my father, Baboucar Niassy, my mother Fatoumata Badji, my adopted mother, Siré Badji, whose love, care, and encouragement have helped me strive to this level. They all never lived to see me through. I also dedicate this work to all my family members for their patience and endurance while I was away for these studies; to my step mother Seynabou Badji who took care of the family after mother passed away; to my elder brother Mamadou Lamine Niassy, my elder sister Hady Djikoher Niassy, my cousins and friends Daouda Diatta, Estella Djitome Kassa Mané (Diatta's wife), Augustin Niassy, and Saliou Djiba; to my uncles El-Hadj Ibrahima Badji, Djissambou Amanga Niassy, Vincent Niassy; and to all my aunts, especially to Famata Adjouberay Kangouye Niassy and Marie Aloumbeuteu Niassy, for their care and encouragement.

ABSTRACT

Cross pheromone-mediating releaser effects between the desert locust, *Schistocerca gregaria gregaria*, and the African migratory locust, *Locusta migratoria migratorioides*, were investigated in olfactometer bioassays. These were compared with responses of gregarious individuals of the two locust species to their own air-borne volatiles.

Similar to previous reports, nymphal and adult stages of *S. gregaria* responded strongly to their own volatiles; immature and mature adults responded to mature adult, but not to nymphal volatiles; nymphs did not respond to mature adult volatiles. The responses of both nymphal and adult stages to their respective volatiles were dose-dependent.

In *L. migratoria*, nymphal and adult stages also responded strongly and in a dose-dependent fashion to their own volatiles. Immature adults responded to volatiles of mature adults, but both immature and mature adults did not respond sufficiently to nymphal volatiles. Nymphs also responded to volatiles of mature adults, but not to those of immature adults.

The two locust species cross-responded to each other's volatiles in a dose-dependent fashion. Both nymphal and mature adult stages of *S. gregaria* were less

responsive to the volatile emissions of the corresponding stages of *L. migratoria*. On the other hand, volatiles from nymphal and mature adults of *S. gregaria* evoked strong aggregation responses in corresponding nymphal and mature adult stages of *L. migratoria*. *S. gregaria* immature adults were more indifferent to volatiles from nymphs of *L. migratoria*, but immature adults of *L. migratoria* were actually repelled by conspecific nymphal volatiles; they further responded poorly to volatiles of nymphal *S. gregaria*.

These results confirm previous findings that in *S. gregaria*, different pheromone systems mediate grouping behaviour in different stages of the locusts. They also suggest that there is an overlap in the pheromone systems mediating grouping behaviour in *S. gregaria* and *L. migratoria*.

The changes in the phase characteristics (primer effects) of nymphal and adult solitarious desert locusts reared mixed with gregarious migratory locusts and the converse, were investigated. Body colour changes, the number of instars and stage duration, pheromone titres (as measured by the amounts of phenylacetonitrile produced by males), morphometrics, and haemolymph pigments composition (as measured by the absorbance ratio at 460 and 680 nm) in test insects were determined. In cage bioassays, significant changes occurred in the phase

characteristics of solitarious nymphs and immature adults of *S. gregaria* which were reared with gregarious nymphs or immature adults of *L. migratoria* with respect to all parameters monitored (though at differerent rates). Similarly, solitarious immature adults of *L. migratoria* which were reared with immature adults of *S. gregaria*, changed significantly in their phase characteristics. Significant changes in phase characteristics also occurred in solitarious *S. gregaria* exposed to volatiles of *L. migratoria*. These findings confirm previous reports that interactions between certain groups of acridids are able to provide the necessary stimuli to initiate locust gregarization (shift from solitarious to gregarious phase).

In another experiment, the effects of gregarious fifth-instar nymphs and mature adults of *L. migratoria* on the sexual maturation of newly moulted gregarious immature males and females of the desert locust, *S. gregaria*, and vice-versa, were investigated by monitoring colour changes and copulation in males, and basal oocyte-length in females. Maturation in *S. gregaria* was signifcantly accelerated by gregarious fifth-instar nymphs of gregarious *L. migratoria*; mature adults did not produce consistent effects. Fifth-instar nymphs and mature adults of *S. gregaria* significantly delayed maturation of newly fledged *L. migratoria*.

Gas-chromatographic (GC) and GC-mass spectrometric analyses of volatiles of similar stages of *S. gregaria* and *L. migratoria* showed quantitative and qualitative differences. In particular phenylacetonitrile was found to be present in the volatiles of nymphal and mature adult *L. migratoria migratorioides*.

The implications of these results are discussed in relation to the behavioural ecologies of the two locust species.

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CHAPTER ONE

1 General Introduction

Locusts are short horned grasshoppers, a large group in the order **Orthoptera.** There are six major locust species in Africa:

- the desert locust, *Schistocerca gregaria gregaria* (F),
- the African migratory locust, *Locusta migratoria migratorioides* (R & F),
- the Red locust, *Nomadacris septemfasciata* (Serville),
- the Brown locust, *Locustana pardalina* (Walker),
- the Moroccan locust, *Dociostaurus maroccanus* (Thunberg), and
- the Tree locust, *Anacridium melanorhodon* (Walker)

The first four species are major agricultural pests in Africa, but the desert locust is regarded as the single most serious pest due to its polyphagous feeding behaviour, and its migratory habits (Popov et al., 1991; Steedman, 1988; Meinzingen, 1993). While the desert locust is widely distributed in Africa, the Middle-East, and parts of Southwest Asia, the African migratory locust is restricted to Africa where it breeds in three ecological zones including the Sahelian, Sudanese and Guinean zones (Zolotarevsky, 1938; Showler, 1995) (Fig.1).

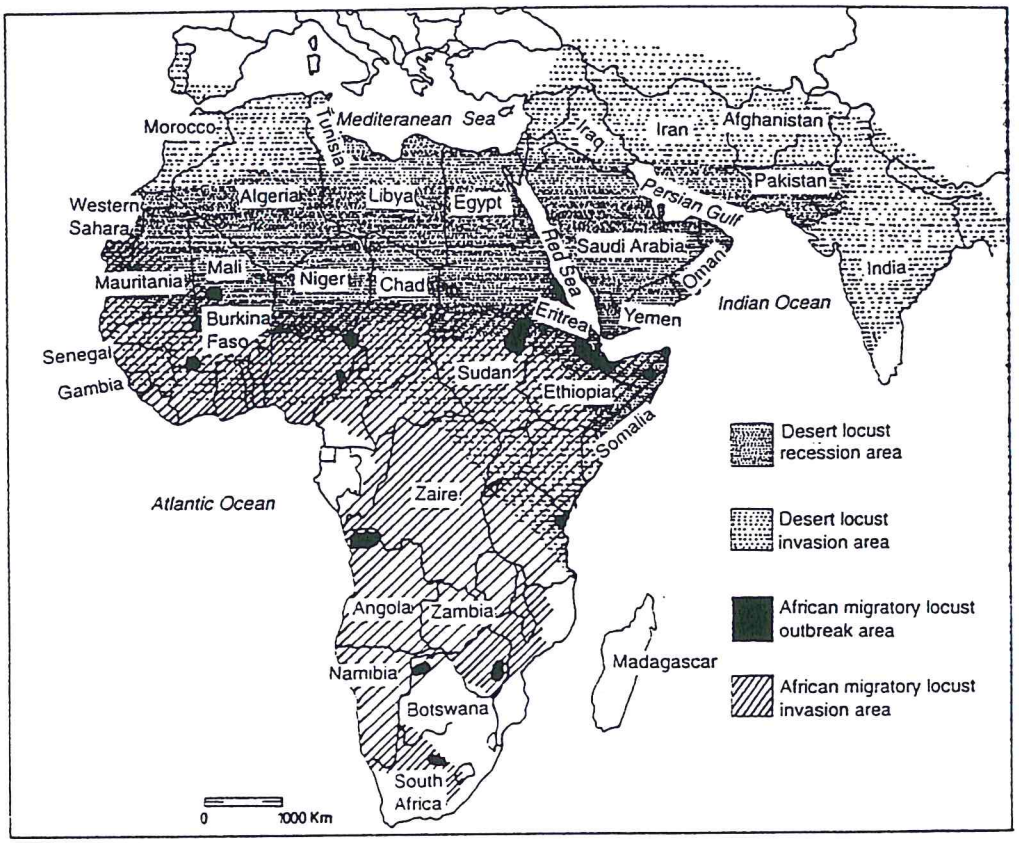

Figure 1. Breeding (recession) habitats and invasion areas of *S. gregaria* and *L. migratoria migratorioides*

S. gregaria and *L. migratoria* both show density-dependent phase polymorphism which refers to the ability of these insects to exist reversibly, depending on density, in two extreme forms or phases: *solitaria* and *gregaria* (Uvarov, 1921; 1923), with intermediate phases known as "*transiens*". The two locust phases differ in behavioural, morphological, and biochemical features, and in fecundity (Uvarov,1921;1923). Between plagues, the two locust species live as solitary, scattered (less than 100 individuals/ha), and harmless individuals in locust affected countries, in areas commonly known as "recession areas" (Fig. 1). When weather conditions(rainfall) become suitable, these individuals concentrate to breed successfully into large numbers, and become gregarious (Roffey and Popov, 1968). Gregarious hoppers do not have developped wings to fly, but they march in dense groups. Gregarious adult stages, however are winged, and therefore, they can swarm over long distances (Descamps, 1961; Duranton and Lecoq, 1990; Steedman, 1988; and Meinzingen, 1993). A typical swarm of *S. gregaria* can be of 300 km^2 in size containing about 40 million individuals, and can travel up to 1000 km a week (Steedman, 1988; Meinzingen, 1993). Swarms of the African migratory locusts are smaller, seldom of similar size, and are usually restricted to their breeding areas (Steedman, 1988).

Locust hopper bands and swarms are very voracious and are, therefore, very destructive. Swarms of the desert locust can cause losses of thousands of tons of crop such as cereals, and vegetables. Meinzingen(1993) reported that damage caused by the desert locust stems from the fact that a locust consumes 1.5 - 3 g of vegetation daily, the equivalent of its own weight. Thus a medium density swarm of 50 million individuals per km^2 can consume 100 tons, and a swarm of 1000 Km^2 can eat 100,000 tons, enough to feed 500,000 people a year (Meinzingen, 1993). However, damage varies with the locust stage and as Meinzingen (1993) had indicated, 8% of the total damage by locusts is due to hoppers, 69% to immature and 23% to mature adults. Direct yield losses from damage due to the desert locust during the plagues of 1954 to 1958, were estimated to vary from 15 million US Dollars in Morocco, 55,000 tons of cereals in Sudan, 16,000 tons of millet in Senegal, and 167,000 tons of cereals in Ethiopia (Steedman, 1988; Popov et al., 1991; Meinzingen, 1993). About 76,000 to 900,000 tons of *sorghum* were lost in the Sahelian Africa during the desert locust plagues from 1986 to 1993, with an estimated maximum cost of control in 1988 of about 102.7 million US Dollars (Herok and Krall, 1995).

The gregarious migratory locust is also polyphagous, but to a lesser extent compared to the desert locust, and

is restricted to graminaceous wild and cultivated plants such as millet, *sorghum*, maize, rice, sugar cane, and bamboo (Meinzingen, 1993). No yield loss assessment exists for this species.

Locust control mainly relies on the use of pesticides. Despite the fact that large scale locust control involves intensive surveys and mapping in order to optimize pesticide use and reduce environmental degradation, the risk of casualities and extensive contamination still exist in locust affected countries (Everts, 1990), because of the fragile ecosystems due to recurrent droughts. Alternative methods including monitoring locust breeding zones for rains and hatchings using remote sensing techniques, the development of gregarization models (Popov et al., 1991), and the use of biological control agents such as entomopathogenic fungi and protozoans (Lomer and Prior, 1992; Thomas et al., 1995), have been proposed. More recently, an integrated pest management approach (El-Bashir, 1991; Joffe, 1995) has been proposed using different, and environmentally sound, packages. It is in this context that the International Center of Insect Physiology and Ecology (ICIPE), launched the Desert Locust Semiochemical Project in 1990 to investigate the semiochemicals that mediate aggregation behaviour, sexual maturation, oviposition, and gregarization of this insect. By the end of 1993,

significant progress had been made by this project in the characterization of these pheromones, and the mechanisms underlying some aspects of phase transformation. However, the project did not include investigations on the role played by other locust species and grasshoppers on the phase dynamics in the desert locust.

To fill this gap, the present study was designed to investigate the interactions between the desert locust, *S. gregaria* and the African migratory locust, *L. migratoria migratotioides* with regard to releaser effects of semiochemicals, and primer effects of all the phase mediating factors.

2 Objectives

2.1 General objectives

The main objective of this work is to establish whether there are interactions between *S. gregaria* and *L. migratoria*, and to investigate releaser effects of semiochemicals and primer effects of the interactions (all phase mediationg factors) on both locust species.

2.2 Specific objectives

To investigate :

1) phase associated releaser effects between and within stages of *S. gregaria* and *L. migratoria;*

2) phase associated primer effects of crowded populations of one species on isolated individuals of the other species in relation to:

   a) the rate of phase transformation in solitarious individuals of the two locust species;

   b) the influence of different stages of crowded locusts on phase change;

   c) the role played by volatiles in these interactions; and

3) cross-effects of crowded live locusts of one species on the rate of sexual maturation of the other.

The procedures used to achieve these objectives are presented in this thesis which is divided into seven chapters. The first chapter contains the general introduction, the second, the review of literature. A general description of the methods and materials used in experimentation follows. Chapters four, five and six deal with studies on locust aggregation (releaser effects), gregarization (primer effects), and maturation

(primer effects), respectively. The last chapter contains two sections comprising a general discussion of the results, and recommendations for future studies. A list of references, and appendices are also provided at the end.

CHAPTER TWO

2 Literature review

2.1 Interactions in insects

Insect species may exhibit diverse types of interactions (Odum, 1971; Price, 1984). Odum (1971) reported that between any two species there could be as many as nine types of interactions including neutralism (neither of the interacting species is benefited), competition (direct interference, and resource use), amensalism (one is inhibited, the other not affected), parasitism, predation, commensalism, protocooperation (non obligatory, but beneficial for both species), and mutualism (both species benefit from their interactions and have become totally dependent on each other for their survival). He added that these can be grouped into negative types of interactions such as predation, parasitism, antibiosis, and interspecific competition; and positive types of interactions including commensalism, cooperation and mutualism (Odum, 1971). To this could be added neutral interactions. Saini and Hassanali (1991) pointed out that in any multitrophic network, a large number of theoretical combinations are possible. For example, between any two levels, six

theoretical categories of interactions are possible depending upon the benefit or harm associated with the signal emitted by a member of interacting pairs (Saini and Hassanali, 1991). Due to the complexity of these ecological relationships, this review is focussed only on examples of recent studies on one and two stage negative, positive and neutral types of interactions.

2.1.1 Negative interactions

Predation and parasitism are examples of interactions between two populations which result in negative effects of growth and survival of one of the interacting populations. Where the two populations have had a common evolutionary history in relatively stable ecosystem, the negative effects tend to be relatively small. Thus natural selection tends to ultimately reduce the detrimental effects and the interactions altogether (Odum, 1971). Severe negative interactions, when coupled with drastic changes in the ecosystem may lead to the extinction of one species and the resurgence of the other. This is probably how pest epidemics (i.e locust or grasshopper invasions) are born, especially when natural ennemies of a species are affected. Predator or parasitoid-prey relationships are the most widely studied interactions because they are of special interest and

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provide an understanding of the mechanisms of host location by pest and predators and/or parasitoids (Saini and Hassanali, 1991). According to Price (1984) , this type is the central issue in understanding both the adaptative strategies for getting food and for avoidance of becoming food. While a predator consumes more than one prey for its survival, a parasitoid survives on a single prey (Price, 1984). Models of predator and/or parasitoid-prey interactions which are developed independently by different authors are used in ecological studies especially in those related to biological control (Price, 1984) . There are as many as three levels of interactions of this type: between the natural enemy species and the host species (carnivore-herbivore); between species of natural enemies (carnivore-carnivore); and between species or host plants, natural enemies, and the herbivore species (plant-carnivore-herbivore).

Carnivore-herbivore

The example reported by Ngi-Song et al.(1995), in which they determined the suitability of the herbivore as food for the carnivore helps to illustrate this level of interaction. These workers found that the exotic *Cotesia flavipes* Cameron preferentially oviposited on larvae of the pyralid species *Chilo partellus* Strand, *Chilo*

orichalcociliellus Strand, *Busseola fusca* (Fuller), and *Sesamia calamistis* (Hampson). However, the local endoparasitoid, *Cotesia sesamiae* Cameron, preferred *S. calamistis*, probably because of some evolutionary adaptation whereby this parasitoid has learned to use this lepidopteran species as a host. They also found that both *Chilo* species and *S. calamistis* were suitable for development of *C. flavipes* with *Chilo* being the preferred hosts, and that *C. sesamiae* preferred *S. calamistis* to the two *Chilo* species. *B. fusca* was not a suitable host for the two parasitoids because of its ability to encapsulate parasitoid eggs in the haemolymph, a form of evolutionary resistance by *B. fusca* to parasitization by this wasp.

Carnivore-carnivore

This level of interaction is well illustrated by the work of Heinz and Nelson (1996) who studied the interactions between the natural enemies of the whitefly *Bemisia argentifolii* (Grenadius)(ex *B. tabaci*), and found that there were no detrimental competitive interactions among the three species of natural enemies studied which would have negatively affected the level of predation and/or parasitism.

Herbivore-herbivore

Coexistence among insects involves spatial interactions which could be viewed to some extent as competition for space resource. The interacting species may learn to coexist for ever or will show competitive periodic displacement (Odum, 1971). This aspect had been studied by Brown et al.(1995) on two aphid species on apple, and found that in such an ecosystem, the Spirea aphid, *Aphis spiraecola* Patch and the apple aphid, *Aphis pomi* De Geer, were similar in their distribution and abundance in late summer when apple is a less suitable host. No significant positive and negative interactions occurred between the two species. *A. spiraecola* reproduced faster to yield more generations through spring to the point that, by the end of the summer, it was the only species present.

Plant-carnivore-herbivore

Recent work on this tritrophic level of interactions include the comprehensive studies by Wiedenmann et al. (1992) involving stemborers such as *Diatrea saccharalis* Dyar, their host plants, and parasitoids such as *Cotesia flavipes*. These studies have revealed some interesting aspects of these interactions especially with regard not

only to the role played by the plant in host location by parasitoids, but also the suitability of the host for survival of the parasitoids.

Abundance of the natural enemies may be favoured by host plant density. Brust et al.(1986) showed that the activity of four most important Carabid predator species of the genera *Pterostichus, Amphasia, Pterosticus, Ambacidus,* and *Harpalus* was more important in no tillage than in tillage corn ecosystem. Thus in no tillage system host plants are more abundant favouring rapid build-up of pest species and consequently, higher populations of their natural enemies. Perfecto et al.(1996) studied the effects of plant diversity and density, on the migration of two ground beetles of the genera *Harpalus* and *Evarthrus.* They found that the emigration rate was higher at low plant density, with negative interactions between the insect migration, plant density, and plant diversity. Thus, emigration was higher in dense polyculture than in dense monoculture. Rupasas et al.(1996) studied in a 4-armed air flow olfactometer the chemical attraction of fourteen *Oryza sativa* Savannah cultivars and one wild *Oryza* species, to the mirid and coccinellid predators of the genera *Cyrtorhinus,* and *Micraspis,* respectively, of the brown planthopper, *Nilaparvata sp.* They found that odours from the plant attracted significantly *Cyrtorhinus* species.

The host plant can, in many forms, resist attack by herbivores, thus enhancing the actions of carnivores. The effects of plant resistance and natural enemies on sawfly were compared by Stein and Price (1995), who found a strong relationship between oviposition preference and larval performance of two tenthredinid sawflies, *Euura sp.*, and *Pontiana sp.* Plant resistance to attacks, in addition to natural enemies, played an important role in the reduction of the sawfly populations.

2.1.2 Positive interactions

Positive association between two species populations are widespread in nature. Positive interactions may be considered as having evolved from commensalism to protocooperation and to mutualism (Odum, 1971). Thus mutualism is the ultimate type of positive interaction, and is discussed in this Section. Symbiotic or mutualistic trophic interactions occur between various species of insects. For example, aphids excrete honeydew consisting mainly of excess ingested sap which is a favourite food for ants (Borror et al., 1976). The ants in turn protect the aphids against predation. Termites, *Nasutitermes corniger* (Motschulsky) obtain nitrogen-rich nutrients and additional nest defence from various species of ants which shelter in their nests during the

wet season (Jaffe et al., 1995). The ants also prey on some live and dead termite workers.

Other forms of somewhat positive trophic interactions include facultative carnivory and/or cannibalism among species, e.g., orthopterans such as *Chrotogonus spp.*, *Aulacara spp.*, *Eyprepocnemis spp.*, and *S. gregaria* (Duranton and Lecoq, 1990; Whitman et al., 1994). In *S. gregaria*, and *L. migratoria migratorioides*, cannibalism is common among fifth-instar nymphs and adults, in *Cataloipus cymbiferus* Krauss, and *Aulacara spp.*, it is common among adults. It has been suggested that this provides a source of necessary proteins to insects that feed on protein-deficient plants (Whitman et al., 1994). This feeding habit has also been documented in many other insect species of the orders Isoptera and Heteroptera (Whitman et al., 1994).

2.1.3 Neutral interactions

Neutral interactions occur when neither of the interacting species are benefited. However, there may never be total neutral interactions unless species are geographically far apart. There are situations in which non related species (in habit and niche) are forced to coexist and with time tend to share some characteristics of survival value such as colour mimicry or semiochemical

signals, without directly affecting each other's life. Such types of interactions are common in grasshoppers, and solitarious or gregarious locusts coexisting in certain breeding areas (Johnston and Buxton, 1949; Steedman, 1988; Showler, 1995). Johnston and Buxton (1949) reported that mixed bands of *S. gregaria* and *L. migratoria migratorioides*, in the Red Sea coastal breeding zones, exhibited marching behaviour characteristic of their gregarious phase. It was not known whether there was change in colour and morphometrics associated with phase change, nor whether they might share the same chemical stimuli. El-Bashir and Abdel-Rahman (1991) later reported some changes in colour and morphometrics in mixed bands of the species. These are discussed in more detail in the following Sections.

2.2 Phase transformation

Phase transformation refers to the ability of locusts to exist reversibly in two extreme phases: solitarious and gregarious. The forms between the two extremes are known as *transiens* (Uvarov, 1921; 1923). Uvarov (1921; 1923) pointed out that all gregarious acridids belong to polymorphic species, and are true locusts. However, some grasshoppers, e.g., *Oedaleus*

senegalensis (Krauss), and *Gastrimargus africanus* Saussure show colour polymorphism and marching behaviour like locusts, but do not swarm nor transform into different phases (Rowell, 1970; Launois and Launois-Luong, 1989). In the family Pyrgomorphidae, some species in the genera *Zonocerus*, *Phymateus*, and *Poikylocerus*, produce aggregation and defence pheromones (Whitman, 1990). However, they do not exhibit reversible phase change, nor form bands and adult swarms like locusts.

Phase transformation is an important trait of locusts which facilitates their survival through migration and recolonization of old habitats and colonization of new areas. It is affected by many factors including, environmental (Steedman, 1988; Popov et al., 1991; Zolotarevsky, 1938), density (Pasquier, 1950; Duranton et Lecoq, 1990, Popov et al., 1991; Deng et al., 1996), chemical stimuli (Nolte, 1963; Gillett, 1975a; Obeng Ofori et al, 1993; Torto et al. 1994; Deng et al., 1996), diet (Jackson et al., 1978), and host and nonhost plants (Steedman, 1988). Interactions with other locusts have also been implicated (Johnston and Buxton, 1949; El Bashir and Abdel-Rahman, 1991).

2.2.1 Interactions with other locusts and grasshoppers

The coexistence of locusts and grasshoppers may be regarded as a neutral type of interaction whereas those between locusts are either mutual inhibitory competition or protocooperation (in accordance with the classification by Odum (1971). Experiments under laboratory conditions have demonstrated that locusts affect phase polymorphism and development of one another when they are crowded in cages while grasshoppers do not (Gillett, 1968; Ba-Angood, 1976). Gillett (1968) studied the grouping behaviour of the desert locust, *S. gregaria*, reared singly with a non-swarming grasshopper species, *Humbe tenuicornis*(Schaum). Isolated locusts and those reared crowded with *H. tenuicornis* were unable to form groups like gregarious populations. She concluded that the grasshopper had no significant effect on the phase status of the desert locust.

In a similar experiment (Ba-Angood, 1976), crowded individuals of *S. gregaria,* and individuals of *S. gregaria* kept among groups of three grasshopper species, *Kraussaria spp.*, *Oedaleus spp.*, and *Aiolopus spp.*, had no significant effect on phase characters such as the number of eye stripes and morphometrics. On the other hand, locust species have been reported to affect phase polymorphism and development of one another when they

occur crowded in the same ecological zones in the field (El-Bashir and Abdel-Rahman, 1991). Johnston and Buxton (1949) documented the occurrence of mixed populations of *S. gregaria* and *L. migratoria migratorioides* whose adults laid eggs together in some breeding areas in the Sudan. They further noted that, within the mixed hopper bands, hatchlings of the two locust species could also be differentiated by their host plant species, and in addition, hatchlings of *L. m. migratorioides* were better swimmers than those of *S. gregaria*. Although effect of the presence of either locust species on the phase characteristics of the other was not investigated, their observations suggest some mutual interactions in addition to overlap of the ecological niches of the two locust species. Gillett (1983) investigated the effects of the fecal volatiles of either *L. migratoria* and *S. gregaria* on each other's gregarization, and found that faeces of the latter affected the melanization and behaviour of the former lending support to Nolte's (1973) observations that these two species produce a common aggregation pheromone (locustol). However, the faeces of *L. migratoria* did not seem to produce the same effects (Gillett, 1983).

El-Bashir and Abdel-Rahman (1991) reported observations of mixed populations of the desert locust and the migratory locust in the Sudan. They observed

that solitarious hoppers of the desert locust which interacted with dense populations of gregarious stages of the migratory locust tended to change into *transiens*. There were increased encounters between hoppers of both species as they marched, roosted, and sheltered under *Zygophyllum simplex* L. They fed together near water courses where the vegetation was greener and soil moisture was conducive to egg-laying. They concluded that the prevailing edaphic factors appeared to have stimulated marching of mixed bands, colour and morphological changes of solitarious *S. gregaria* towards the transient phase. Such observations were confirmed by Torto in 1995 (personnal communication) in the locust breeding areas along the Red Sea coast in the Sudan. The observations by these authors did not involve analyses of parameters such as locust body colour, morphometrics, marching behaviour, number of eye stripes, and pheromone production. Therefore, detailed studies on the interactions between the desert locust and the migratory locust are lacking.

2.2.2 Environmental conditions

Environmental factors, especially rainfall play a significant role in the phase transformation of both the desert locust and the African migratory locust

(Zolotarevsky, 1938; Ellis and Carlisle, 1965; Carlisle and Ellis, 1965; Popov et al., 1991). Adequate rainfall supports the growth of annual plants and shrubs which when fed on by immature solitary adults hasten their sexual maturation. This is followed by congregation of individuals at suitable sites for mating and egg laying. Uvarov (1966), Rowell (1970), and Fuzeau-Braesch (1972) have shown that in locusts, environmentally regulated phenotypic colour polymorphism such as homochromy, green brown polymorphism, and density dependent polymorphism may be found. Faure (1942), Zolotarevsky (1938), and Albrecht (1964) showed that solitary *L. migratoria* were green under high humidity, but in low humidity they were near brown in colour. Furthermore, they observed that under low humidity, solitary hoppers adjusted their colour from whitish-cream, yellow, brown, grey, or black to match the colour of the background environment they were in. Zolotarevsky (1938) studied the effects of humidity on the ratios of lengths of the elytron to the femur (E/F), and the length of the femur to the width of the head capsule (F/C) of the migratory locust. He found that dry conditions (RH $\leq$ 45%) tended to favour gregarious characters as compared to wet conditions (RH $\geq$ 70%). Increasing humidity coupled with increasing daylength and temperature favoured solitarious

morphometric ratios in *L. migratoria* (Albrecht and Lauga, 1978; 1979).

Temperature has an effect on nymphal displacement behaviour in *L. migratoria* (Descamps, 1961). When temperatures are high (above 30°C) nymphs tend to find shelter by hiding under shrubs, trees or roost on top of grasses (Descamps, 1961: Steedman, 1988). At very low temperatures (below 26°C), they tend to find cover under dead leaves, and shrubs. This facilitates grouping of solitarious nymphs especially in patchy vegetation, and may lead to gregarization of nymphs if they are in contact with each other for sufficiently long periods (Bouaichi et al., 1995). Colour patterns of locust cuticle also vary with temperature. Field and laboratory observations (Stower, 1959; Dudley, 1964), showed that at low temperatures the black patterns were predominant on locust hoppers while at high temperatures they were fewer. Morphometrics of adults were found to change significantly with temperature. At a temperature of about 30°C and relative humidity of about 90%, locusts tended to shift toward gregarious E/F ratios. On the other hand, temperatures greater than 30°C, and at the same relative humidity of 90%, E/F ratios of individuals were characteristic of solitarious phase. F/C ratios only changed at relatively lower humidity (50%). Stower (1959), and Meinzingen (1993) showed that at high

temperature (above 30°C) gregarious desert locusts showed morphometrics which were similar to those of solitarious locusts reared at temperatures below 30°C. Thus there is ecological relative fitness of locusts to their environment as manifested by the changes in morphometric and colour, in relation to changing condtions.

Habitat patchiness also plays an important role in phase dynamics, especially during the dry season when host plants are found in patches throughout the habitat. It is more evident in the case of the migratory locust in the breeding areas of the Niger (Lean, 1931; Zolotarevsky, 1938; Steedman, 1988) where individuals are forced to concentrate on green patches of vegetation resulting from previous floods. Grouping of solitary adults is then facilitated, and if conditions are conducive, synchronous egg-laying occurs leading to synchronous egg development and hatching (Roffey and Popov, 1968).

2.2.3 Density

Gregarization is mainly a density-driven process (Pasquier, 1950). Under favourable environmental conditions (e.g. sufficient rainfall and vegetation) adult locusts mature faster, copulate, and gravid females concentrate at suitable sites for egg-laying. The

attraction of such females to egg laying sites is favoured by adequate soil moisture and froth volatiles from deposited eggs (Saini et al., 1995). Gregarized females lay eggs which yield gregarious nymphs, a phenomenon known as "maternal inheritance" (Hunter-Jones 1958; Injeyan and Tobe, 1981; Bouaichi et al., 1995; Islam et al., 1995). Furthermore, grouping of nymphs that hatched together favoured prolonged contact and emission of aggregation pheromones (Deng et al., 1996), which could keep nymphal groups together. In addition, at high densities tactile, visual, olfactory, and acoustic interactions play a role in maintaining the gregarious phase. Nymphs hatching together form small cohesive groups at the early stage, feed from plant to plant, and keep merging with other small groups in their paths into larger groups (Steedman, 1988; Meinzingen, 1993). This progressive increase in density was referred to by Pasquier (1950) as "densation". Roffey and Popov (1968) and Duranton and Lecoq (1990) described three stages (multiplication, concentration, and gregarization) in the process leading to the build up of locust populations in the field, and which also influence phase shifts. They observed that in the Tamesna mountains breeding area in the Niger, the multiplication factor (ratio between parental and actual density) was estimated at 16 - 20 fold. Popov et al. (1991) suggested

that the actual multiplication factor in such situations could be about 100 - 200 fold. Population build up could also be facilitated by active immigration, e.g. through attraction (Pedgley, 1981), or passive immigration e.g. by random effects of wind (Roffey, 1969; Popov, 1969; Shaeffer, 1972). Hunter-Jones (1958) studied the inheritance of phase characters in hoppers and adults of *L. migratoria*, and *S. gregaria*. By monitoring hopper colour patterns, weight of hatchlings, number of nymphal instars, and adult colour and morphometrics, he found that hoppers reared in isolation appeared green similar to solitarious locusts. Morphometric ratios (E/F and F/C) also corresponded to those of solitarious insects. He correlated weights of hoppers of *S. gregaria* with colour or adult morphometrics and found that, the green hatchlings (solitary) had relatively higher morphometrics and lower weight compared to their black and yellow counterparts (gregarious). Nolte (1973) showed that crowding at high densities in the field and in the laboratory increased melanin deposition, chiasma frequencies in *L. pardalina*, and *L. migratoria* while transient morphometrics occurred in *S. gregaria*. Crowding of the grasshopper *Paracinema tricolor* Thunberg had no effect on chiasma frequency (Nolte, 1973).

Deng et al. (1996) studied the effects of shifting *S. gregaria* from crowded to isolated conditions and vice-

versa on the emissions of adult aggregation pheromones in the laboratory, and compared this with changes in morphometrics. They demonstrated the extreme sensitivity of the desert locust to crowding, such that even pairing two solitarious locusts provided each other with the necessary stimuli to cause their gregarization. Further, they showed that adult males of the parental generation resulting from shifting crowd-reared hoppers, immature or mature adults to isolated conditions did not produce phenylacetonitrile similar to solitary-reared adults. Conversely, adults of the parental generation resulting from shifting solitary-reared hoppers, fledglings, or mature adults to crowded conditions produced pheromone at levels which were similar to those of control adults from the crowd-reared colony. In contrast, they showed that morphometric changes were slow and required several generations to show significant variations, confirming earlier findings by Chapman (1979). Also, the F/C ratio (length of the femur to the width of the head capsule) was shown to be more sensitive to treatment effects than the E/F ratio (length of the elytron to the length of the femur).

2.2.4 Chemical stimuli

Nolte (1963) was the first researcher to demonstrate the mediation of a chemical stimulus in the gregarization of locusts. He reported the presence of a chemical factor in the air-borne volatiles of crowded locusts which acted as a "primer" pheromone facilitating the grouping of locusts reared in isolation. Gillett (1968) confirmed the existence of such a chemical stimulus in the air-borne volatiles of crowded locusts. Nolte referred to this as a "*gregarization pheromone*" which was later identified as 5-ethyl guaiacol in the nymphal faeces and was called "*Locustol*" (Nolte, 1970). Locustol evoked such gregarious characteristics in crowded locust populations as increase in chiasma frequency, melanin pigmentation, and changes in adult morphometric ratios towards those of phase *gregaria*. More detailed studies by Nolte (1973) suggested that three locust species, *S. gregaria, L. migratoria,* and *L. pardalina* produced, and utilized locustol for gregarization. The red locust, *Nomadacris septemfasciata* (Serv), the Australian locust, *Chortoicetes terminifera* Walk., and a grasshopper *Paracinema tricolor,* did not demonstrate evidence of the production of this pheromone (Nolte, 1973).

Studies by Gillett (1975) showed that the aggregation pheromone mediated grouping behaviour in

gregarious nymphs and it was not produced by isolated nymphs in quantities that could alter each other's behaviour. She discussed two sets of pheromones, a gregarizing pheromone which emanated from faeces of crowded nymphs, and a solitarizing pheromone, only detectable in the faeces of crowded adults (Gillett and Phillips, 1977). A more detailed comparative study on the pheromonal systems of *S. gregaria* and *L. migratoria migratorioides* was conducted by Fuzeau-Braesch et al.(1988). They detected four volatile aromatic compounds in the environment surrounding *S. gregaria, L. migratoria migratorioides, and Locusta migratoria cinearensis* (Fabricius) of which three were identified as phenol, guaiacol, and veratrole. Phenol, guaiacol, and the blend of the three compounds were found to act as cohesion pheromones for the first two locust species, while *L. m. cinearensis* did not produce detectable amounts of these pheromones.

Recently, detailed work relating to releaser pheromones mediating aggregation behaviour in the desert locust revealed that a complex of pheromones produced by different locust stages and sexes, and in their faeces are involved (Obeng-Ofori et al. 1993; 1994a;1994b; Torto et al., 1994; 1996). The pheromone systems mediating aggregation behaviour in nymphal and adult stages of *S. gregaria* were charaterized by Torto et al.(1994; 1996)

using a more efficient volatile collection technique. They identified six electrophysiologically active compounds in the volatiles of adult males of the desert locust comprising anisole, benzaldehyde, veratrole, guaiacol, phenylacetonitrile, and phenol. Veratrole, guaiacol, and phenol which were previously detected by Fuzeau-Braesh et al.(1988), were also present, but in minor amounts in the atmosphere surrounding fifth-instar nymphs and adults of *S. gregaria*. Of the six compounds, only four compounds phenylacetonitrile, benzaldehyde, guaiacol, and phenol were found to elicit aggregation responses (Torto et al., 1994). Phenylacetonitrile was found to be a major and key component since it elicited aggregation responses in adults similar to the crude extracts. Furthermore, it was shown that only gregarious mature adult male locusts produced the aggregation pheromone, and that solitary locusts responded to it (Torto et al., 1994; Njagi et al., 1996). It was suggested that, male aggregation pheromone may play a role in the 'arrestment' and recruitment of solitary individuals into gregarizing or gregarious groups in the early stages of locust outbreaks (Njagi et al., 1996).

Subsequently, it has been shown that production of phenylacetonitrile by gregarious mature adult male desert locusts follows a sigmoidal pattern, starting in the first 10 days after fledging, peaking between 15 and 20

days, and leveling off by the 35th day after which it starts to drop to reach very low levels in very old adults (Torto et al., 1994; Deng et al., 1996; Assad et al., 1996). Deng et al.(1996) showed that pheromone titres (as measured by the amounts of phenylacetonitrile) are a more sensitive measure than morphometrics in determining the onset of phase change in *S. gregaria*.

The nymphal pheromone system of gregarious *S. gregaria* was characterized as consisting of three sets of compounds: aliphatic C_6,C_8-C_{10} aldehydes and their corresponding acids produced by the insects themselves, augmented by faecal phenols, guaiacol and phenol (Torto et al., 1996).

2.2.5 Dietary factors

Locusts use their host plants for food and/or shelter. The effects of diet from such plants as alfalfa, hedge mustard, Johnson grass, and the mixture of the three, on the lesser migratory grasshopper, *Melanoplus mexicanus mexicanus* (Saussure) were studied by Barnes (1955). He monitored many parameters of which nymphal development and weight, and adult body dimension ratios (morphometric) showed significant diet-dependent variations. Adults reared on hedge mustard and mixed diet showed traits towards the migratory phase, those

reared on Johnson grass were intermediate whereas those reared on alfalfa showed little or no development toward the migratory phase. Toye (1973) studied the effects of five different food plants on the development of the desert locust, *S. gregaria* and found that hoppers fed on *Agropyron* and *Poa* grasses showed the highest developmental rate of nymphs (29 - 32 days). The heaviest nymphs and adults had morphometrics typical of the gregarious phase. When fed on lime, privet, and spinach they produced morphometrically smaller and less viable males which had some abnormalities in nymphal development (five instars with a long fourth and without the fifth instar). Jackson et al.(1978) investigated the effects of natural food plants on the phase of the desert locust, *S. gregaria* using cultivated plants *Pennisetum typhoides*(Burm.f.) and *Sorghum sp.* and the desert host plant *Dipterygium glaucum* Oecn. *Tribulus spp.*, *Chrosozophora oblongifolia*(Del.), *Panicum turgidum* Forsk. and *Zygophyllum simplex* L. They found that a diet of *Pennisetum,* and *Sorghum* tended to enhance gregarious traits while *Dipterygium* accentuated solitarious characteristics. This was also apparent on the progenies of parents fed on these plants. Mishra and Singh (1992), Langewald and Schmutterer (1992), Schmutterer et al.(1993), and Doumbia (1994) found that feeding on, or topical application of, neem (*Azadirachta indica* L.)

extracts to nymphal and adult locusts tended to reverse their phase from gregarious to solitarious.

2.3 Methods of characterizing phase in locusts

The phase status of *S. gregaria* and *L. migratoria* has commonly been characterized on the basis of morphometric ratios (Uvarov, 1921; 1923), behaviour (Ellis, 1962), body colour (Stower, 1959), physiological characters such as eye colour (Stower, 1959), eye stripes (Uvarov, 1966), and fecundity as expressed by the number of ovarioles, eggpods, and eggs per pod (Norris, 1950; Albrecht et al., 1958; Hunter-Jones, 1958; Papillon, 1960; Injeyan and Tobe, 1981a), haemolymph pigment composition (Mahamat et al., 1996), and pheromone emission (Torto et al., 1994). These phase charateristics are discussed below.

2.3.1 Morphometrics

Morphometric measurements were one of the first methods used to separate phases (Uvarov, 1921). It was established that *Locusta danicus* Linnaeus was the same as *L. m. migratorioides*, and this was the basis of Uvarov's phase theory. Uvarov (1923) used the morphometric E/F ratio {ratio of the length of the elytron (E) to that of

the hind femur (F) }, to separate the solitarious and the gregarious phases of *S. gregaria*. Dirsh (1953) used the F/C ratio { ratio of the length of the hind femur (F), to the width of the head capsule (C) }, and found that it was a more reliable phase parameter than the E/F ratio. The E/F and F/C ratios of *S. gregaria* and *L. migratoria* are summarized in Table 1.

Table 1. E/F and F/C ratios of *S. gregaria* and *L. migratoria* (Meinzingen, 1993)

Locust species	Phase	E/F males	F/C males	E/F Females	F/C Females
S.gregaria	*solitaria*	<2.075	>3.75	<2.075	>3.85
S.gregaria	*gregaria*	<2.23	<3.15	<2.27	<3.15
L.migratoria	*solitaria*	<1.83	3.67	<1.83	3.46
L.migratoria	*gregaria*	<2.0	2.96	<2.09	2.86

A method by which locust phase could be determined using both E/F and F/C ratios in a chart was proposed by Rungs (1954) and later modified by Duranton and Lecoq (1990) for more practical use in the field. It allows quick phase determination in a locust population. The proportion of any locust population of interest which undergoes phase shifts in the test population could be quickly determined. However, Chapman (1979) and Deng et al.(1996) suggested that morphometric parameters should

be considered with caution for phase description since they change slowly over several generations.

2.3.2 Body colour

Body colour patterns can be valuable for monitoring phase shift from the solitary to the gregarious phase, and vice-versa. Nymphal stages of the solitarious desert locust are often characterized by green straw colour and adult stage by greyish brown (Pener, 1991). In the gregarious phase, nymphs have a black pattern on a yellow background (Nickerson, 1956; Stower, 1959; Pener, 1991) while adults are pinkish when immature and bright yellow when mature (Norris, 1952, 1954). Stower (1959) and Duranton and Lecoq (1990) classified colour stages in nymphal desert locusts from 0-5, based on the extent of the black emaculation on the head and other body parts. A grade of 0 corresponded to no black markings on either the head, hind femora, pronotum, or the abdominal tergites, while grade five corresponded to full black markings on these body parts. Norris (1954) used a colour classification scheme made of five grades for adult *S. gregaria*. Grade I corresponded to pink immature adults, with no flush of yellow at all on abdominal tergites and hindwings, grade III denoted adults with a flush of yellow on abdominal tergites, wings, and thorax,

and grade V corresponded to full bright yellow colour in fully mature adults. This classification was modified slightly by Loher (1960) into four stages to monitor the acceleration of maturation in the desert locust.

In *L. migratoria*, the solitarious adult locusts are green or brown while gregarious nymphs are black with an orange ventrum (Faure, 1942; Gunn and Hunter-Jones 1952; Hunter-Jones, 1958). Hunter-Jones (1958) described six colour types in hatchlings of *L. migratoria* : type 1 corresponded to pale brown pattern on a yellow brown ground colour, type 3 a medium to dark brown covering on one third of the body surface, and type 6 to black hatchlings with little to no apparent ground colour. No such classification has been proposed for adults.

2.3.3 Eye stripes and shading

The number of eye stripes in *S. gregaria* and *N. septemfasciata* has been correlated with the number of hopper instars in crowded and isolated hoppers (Uvarov, 1966). In *S. gregaria*, solitarious hoppers have seven stripes corresponding to six nymphal instars whereas the gregarious hoppers have six showing nymphal development through five instars (Uvarov, 1966). Stower (1959) and Duranton and Lecoq (1990) showed that eye colouration could also be a phase marker in the desert locust. They

found that while solitarious locusts had clear eyes with all the stripes visible, gregarious ones tended to have totally darkened eyes. Based on this, they graded the eye colouration from 0-5 as follows: 0, no shading of the eye; 1, one third of the eye shaded black; 2, one half shaded black; 3, one half shaded with additional black spots on the other half; 4, two thirds shaded with additional spots; and 5, eyes fully coloured black.

2.3.4 Haemolymph pigments

Mahamat et al.(1996) studied the presence of the blue biliverdin pigments in the haemolymph of *S. gregaria*. They showed that these pigments are associated with all stages of and ages of the solitary-reared (solitarious) insects, irrespective of their origin or diet, but absent or present in very small amounts in the crowd-reared (gregarious) ones. The ratio of absorbance at 460 nm (max for the carotenoids) and 680 nm (max for the biliverdin pigments) in a UV spectrophotometer was found to be a convenient and reliable phase marker. Such ratio falls in the range of 3.99 - 4.78 in the gregarious and from 0.64 - 1.67 in the solitarious locusts (Mahamat et al., 1996).

2.3.5 Pheromones

The use of aggregation pheromones as a phase marker was recently demonstrated by Torto et al.(1994) and confirmed by Deng et al.(1996) by comparing pheromonal emissions of gregarious and solitarious adult desert locusts. Only adult mature male gregarious locusts were able to produce aggregation pheromone (Torto et al., 1994; Obeng-Ofori et al., 1994a). Phenylacetonitrile is the major and key compound in the pheromone system of gregarious adult *S. gregaria*. Deng et al.(1996) showed that pheromone titres (as measured by the amounts of phenylacetonitrile) represent a more sensitive measure than morphometrics of the onset of phase change in *S. gregaria*.

2.4 Developmental life cycle

The developmental cycle of both *L. migratoria* and *S. gregaria* comprises three stages: egg, nymph, and adult (Steedman, 1988; Meinzingen, 1993) (Fig. 2). Eggs are deposited by mature females in the soil where the whole embryonic development occurs. At the completion of the embryonic development, the eggs hatch and neonates crawl

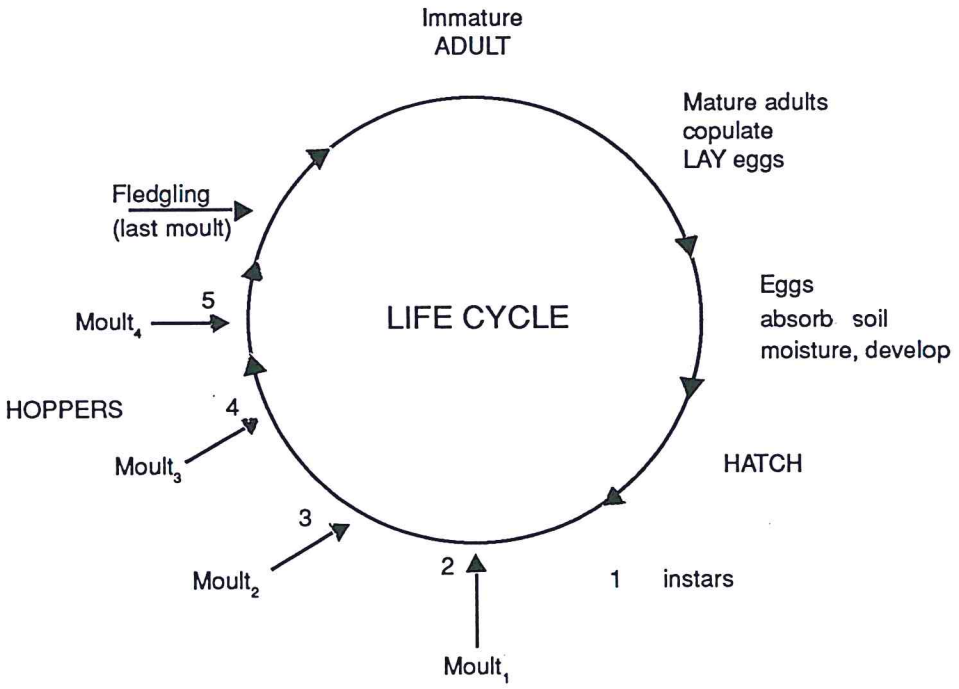


Figure 2. Life cycle of gregarious desert locust and migratory locust (Steedman, 1988) (solitary *S. gregaria* and sometimes *L. migratoria* have a sixth-instar)

their way up to the ground surface. Nymphs develop through five to six instars depending on their phase and species before fledging into adults (Uvarov, 1966; Steedman, 1988; Meinzingen, 1993). The adult develops through two stages, a sexually immature stage corresponding to pink coloured adults and which do not copulate nor oviposit (Norris, 1954), and a mature stage corresponding to yellow coloured and sexually mature locusts which copulate and oviposit (Popov, 1954; Norris, 1954). This developmental life cycle is reviewed in more detail in the following sections.

2.4.1 Egg development

Eggs are laid in the soil where, under favourable conditions of soil humidity and temperature, they take, on the average, 15 days to incubate. Their development is affected by environmental conditions. For example, Ackowor and Vajime (1995) found that in the lake Chad breeding area of *L. migratoria migratorioides*, egg development is closely related to season, soil structure, and natural enemy complex in the area. The fastest egg development was recorded during the main rainy season in sandy soil at temperatures of around 29°C with an incubation period of 13.6 days while the slowest was recorded during the Harmattan (dry) season at

temperatures ranging from 19°C to 26°C. Incubation time was 24 days in clayey soils.

No distinct diapause has been observed in the egg development in both *S. gregaria* and *L. m. migratorioides*, even though Meinzingen (1993), and Akowor and Vajime (1995) showed that in the field, some slowing or temporary arrest of the egg development is possible in conditions of low soil humidity and temperature conditions. In *L. migratoria*, the incubation period ranges between 20 - 40 days in cold conditions, but only 10 - 20 days in warm conditions (Price and Brown, 1990; Meinzingen, 1993).

The longer incubation time is a quiescent period (quiescens) and development and hatching proceed normally as soon as conditions become favourable.

2.4.2 Nymphal development

Nymphal developmental time varies from 24 - 57 days depending on such conditions as temperature and humidity, and on locust phase. Under conditions of optimum temperature and humidity, gregarious locusts develop faster than solitarious ones (Duranton and Lecoq, 1990). In *S. gregaria*, nymphal development goes through five instars over a period of 25 days in the gregarious phase, and through six nymphal instars in a period of 30

days in the solitarious phase. Under adverse conditions, development may take 50 days in the gregarious phase and up to 90 days in the solitarious phase (Duranton and Lecoq, 1990). In *L. migratoria*, nymphal development goes through five instars regardless of the phase, over a period of 24 to 35 days. However, under adverse conditions, solitarious individuals may go through six or seven instars and development could last as long as 60 days (Meinzingen, 1993).

2.4.3 Adult sexual maturation

At the end of the last instar, nymphs fledge into sexually immature adults. When water and food are adequate, the immature adults of *S. gregaria* become sexually mature, copulate and oviposit in about 18 to 30 days (Steedman, 1988; Duranton and Lecoq, 1990). A mature adult female can lay 2 to 3 eggpods containing 30-70 eggs each when gregarious, and more than 3 eggpods containing 55-140 eggs each, when solitary (Steedman, 1988; Duranton et Lecoq, 1990). In *L. migratoria*, this number varies from 55-140 with an average of 67 eggs/pod (Steedman, 1988; Meinzingen, 1993) in the solitarious phase, and an average of 39.4 eggs/pod in the gregarious phase (Meinzingen, 1993). The time from egg to first oviposition in *S. gregaria* can vary from 50 to 332 days,

depending on soil moisture, relative humidity and temperature conditions, and locust phase status (Duranton and Lecoq, 1990). In both species adult longevity varies from 75 to 150 days.

On the average, *S. gregaria* may go through 3 generations (1-5 generations) a year (Steedman, 1988; Meinzingen, 1993) depending on how fast the immature adults find suitable breeding conditions. Also, *L. migratoria* can produce up to five generations per year in the main outbreak area, but only two elsewhere in Africa (Meinzingen, 1993).

Adult sexual maturation is affected by such factors as rainfall, humidity, temperature (Norris, 1954; 1957; Steedman, 1988), chemical stimuli (Norris, 1954, 1968; Loher, 1960; Mahamat et al., 1993; Assad, 1995), host and nonhost plants (Jackson, 1978; Assad, 1995).

2.4.4 Rainfall, humidity, and temperature complex

When rainfall, humidity, and temperature are not adequate, immature adult locusts undergo arrested sexual maturation known as quiescence or imaginal diapause (Meinzingen, 1993). In *S. gregaria*, this state can last as long as seven months (Duranton and Lecoq, 1990). When conditions are favourable, sexual maturation proceeds. Rainfall leads to growth and germination of nutritious

vegetation that supports rapid development (Carlisle and Ellis, 1965) and sexual maturation in immature adults. This leads to mating and egg laying both in solitary and gregarious locusts (Steedman, 1988; Meinzingen, 1993). Duranton and Lecoq (1990) and Meinzingen (1993) reported that at low temperatures (17°C and below), locusts tend to slow their maturation even if rainfall was adequate. Maturation is equally slowed down at high temperatures (above 30°C) in the absence of rainfall, but accelerated at temperatures between 27-30°C and adequate rainfall 20-100mm (Steedman, 1988; Duranton and Lecoq, 1990).

2.4.5 Chemical stimuli

Pheromones play an important role in locust maturation and is particularly effective in inducing yellowing in males and females, testicular and ovarial development in male and female maturing locusts, respectively (Loher, 1960). Norris (1964) studied the accelerating and inhibiting effects of crowding on sexual maturation in two locust species, *L. m. migratorioides* and *S. gregaria*, and found that crowding accelerated maturation in the latter, but delayed it in the former. She further showed that young adults exerted inhibiting effects whereas older adults exerted accelerating effects on maturing adults. She concluded that the depression of

activity is a response to some non-mechanical, non-visual, and presumably, a chemical stimulus which accelerated maturation in males and stimulated ovarial development and readiness to copulate in females of *S. gregaria*.

When immature adults of the desert locust, *S. gregaria,* were exposed to themselves, they tended to copulate after 28 days, however when they were exposed to mature males most of them copulated after 17 days (Loher, 1990). In the migratory locust, *L. migratoria migratorioides* the acceleration was from 17-25 days to 13-14 days (Loher, 1990). In the absence of mature adult locusts maturation is delayed in *S. gregaria* (up to 28 days), but in *L. migratoria*, it is accelerated to only 7 days after final molt (Loher, 1990). Norris (1964) observed that young adults of *L. migratoria* in crowded conditions tended to inhibit each other from maturing over a certain period. The nature of the inhibition was not identified. In *S. gregaria*, it was the opposite, isolation preventing pheromone production (Njagi et al., 1996; Deng et al., 1996). In a recent study, Mahamat et al.(1993) applied the colour grading scale of Norris (1954) and pheromone release in gregarious adults described by Torto et al.(1994) to study maturation-acceleration in immature *S. gregaria*. They observed that the compounds identified by Torto et al.(1994),

especially phenylacetonitrile, were the ones responsible for hastening maturation of immature males and females. Assad et al. (1997) investigated the effects of the nymphal stage of *S. gregaria* on the maturation of immature adults. Sexual maturation was significantly delayed in adults that were exposed to volatiles of fifth-instar nymphs. They further showed that live male and female nymphs placed in an upper compartment of two chamber cages were equally effective in inducing this delay. However, their faeces were ineffective, suggesting the mediation of a volatile signal from the nymphs themselves. Nymphal volatiles containing C_6, C_8-C_{10} aldehydes and acids, phenol, and guaiacol which are nymphal aggregants (Torto et al., 1996), also act as maturation retardants for young adults of *S. gregaria* (Assad et al., 1997).

2.4.6 Interactions between species

Norris (1964) studied sexual maturation in *S. gregaria* and *L. migratoria* and reported that mature adult *S. gregaria* accelerated sexual maturation of young adults of *L. migratoria*. It was not clear whether mature adults of *L. migratoria* had the same effects on immature adults of *S. gregaria*.

2.4.7 Host and nonhost plants

Carlisle and Ellis (1967) studied the synchronisation of sexual maturation in desert locust swarms and found that locusts fledging at the beginning of the dry season and feeding on senescent vegetation failed to mature. This was attributed to the low concentrations of giberrellin and the monoterpenoid eugenol in their diet confirming their earlier findings (Ellis and Carlisle, 1965). Maturation occurred in young adults which fed on green leaves containing these substances at the onset of the rainy season. Sexual maturation was also affected by odours of essential oils from certain desert shrubs (Carlisle and Ellis, 1967).

Jackson et al.(1978) showed that different food plants variably affected many aspects of locust growth and development. For example, *Pennisetum, Dipterygium, Tribulus, and Chrozophora* supported rapid growth and synchronized moulting. On the other hand, hoppers reared partly on a pure diet of sorghum completed their nymphal development, but many were retarded with poor moulting synchronization and low body weight. Sexual maturation was adversely affected.

Price and Brown (1992) found that migratory locusts had a high reproductive performance in maize monoculture during summer and in wheat monoculture during spring

seasons. They suggested that green food was essential for continuous reproduction, and that drying out of food plants could stop reproduction even at the oviposition stage, forcing locusts to enter into reproductive quiescence or delayed maturation.

Effects of a desert plant of the genus *Commiphora* on the sexual maturation of the immature males and females of the desert locust were investigated by Assad et al.(1997). They found that extracts (containing 55 detectable compounds mainly terpenoids) from this plant, when collected before winter rains accelerated sexual maturation of immature locusts. However, when collected after the winter rains no such acceleration was observed. Thus, volatiles from the host plant affect sexual maturation in locusts, and the effects vary with seasons (Carlisle and Ellis, 1967; Assad et al., 1997).

2.5 Methods of characterizing sexual maturation

2.5.1 Integumental colour

Maturing adults of *S. gregaria* become progressively yellow according to Norris (1954) and Loher (1960) colour classifications (Section 2.3.2 of this thesis). Such a scheme has not been applied in *L. migratoria* even though

in both locusts, yellowing is a sign of sexual maturation (Norris, 1950; 1964; Loher, 1990; Whitman, 1990).

2.5.2 Copulation

Norris (1950; 1954) found that mature males of *S. gregaria*, and *L. m. migratorioides* copulated as soon as they became sexually mature. This was applied later by Mahamat et al.(1993), and Assad (1995) and Assad et al.(1997) to study maturation acceleration and retardation, respectively, in the desert locust, *S. gregaria*.

2.5.3 Pheromone emission

Emission of the aggregation pheromone was used by Mahamat et al. (1993) and Assad (1995) to study sexual maturation of the desert locust, *S. gregaria*. They showed that, pheromone emission is also a sensitive indicator of sexual maturation since maximum pheromone production in males coincides with age at full maturity.

2.5.4 Length of basal oocytes and oviposition

The length of basal oocyte was used as an indicator of sexual maturity in studying the rate of maturation of

immature adult females of *S. gregaria*, since this indicates the rate of ovulation (Norris, 1954). Norris (1954) showed that oocyte length between 6 and 8mm corresponded to sexual maturity in females. Descamps and Wintrebert (1961) defined four maturity stages in female migratory locusts, *L. m. migratorioides*. Locusts in stage III maturity or reproductive stage had oocyte lengths of 3-7 mm. Highnam and Haskell (1964) studied the relationship between the endocrine system, flying activity and oocyte lengths in both *S. gregaria* and *L. m. migratorioides*. They showed that in *S. gregaria*, the oocyte lengths varied from 0.7 mm in fledglings to 8.0 mm in fully mature adults. In *L. m. migratorioides* the lengths varied from 0.7 mm in fledglings to 6.5 mm in fully mature adults. They further showed that the variations in oocyte lengths were also influenced by the locust phase as in isolated *S. gregaria*, and crowded *L. m. migratorioides*, oocyte growth was slow, but in crowded *S. gregaria* and isolated *L. m. migratorioides*, oocyte growth was faster. There was a strong correlation between the variations in the lengths of oocytes and the size of the *corpora cardiaca*, on one hand, and the amount of neurosecretory material released by these glands in the *pars intercerebralis* of the brain, on the other (Highnam and Haskell, 1964).

The time to first oviposition is also a useful parameter in determining sexual maturation of the desert locust (Norris, 1950; 1954; Mahamat et al., 1993).

CHAPTER THREE

3 General materials and methods

3.1 Insects

Crowded desert locusts, *S. gregaria* were obtained from a colony maintained at the ICIPE. The colony was propagated (for seventeen generations by the beginning of this work), from an original stock which was obtained from the Desert Locust Control Organisation for East Africa (DLCO-EA) in Addis Ababa, Ethiopia (Ochieng-Odero et al., 1991). Crowded migratory locusts were obtained from a stock maintained (for 16 to 17 generations at the beginning of the experiments) at the University of Nairobi, Kenya, and propagated for the experiments.

To keep the colony gregarious, locusts were reared crowded (100-200 nymphs or 60-100 adults) in aluminium cages of 50 x 50 x 50 cm (Fig. 3A; Plate H) whereas for a solitarious stock, locusts were reared individually in 10 x 10 x 15 cm aluminium cages (Fig.3 B)(Ochieng-Odero et al., 1991). The rearing room (dimension: 2.5 x 2 m) was maintained at 29 ± 2°C., 58 ± 1 % RH, and 12:12 H light:dark period. The room was aerated by a duct system which maintained a negative pressure that facilitated a

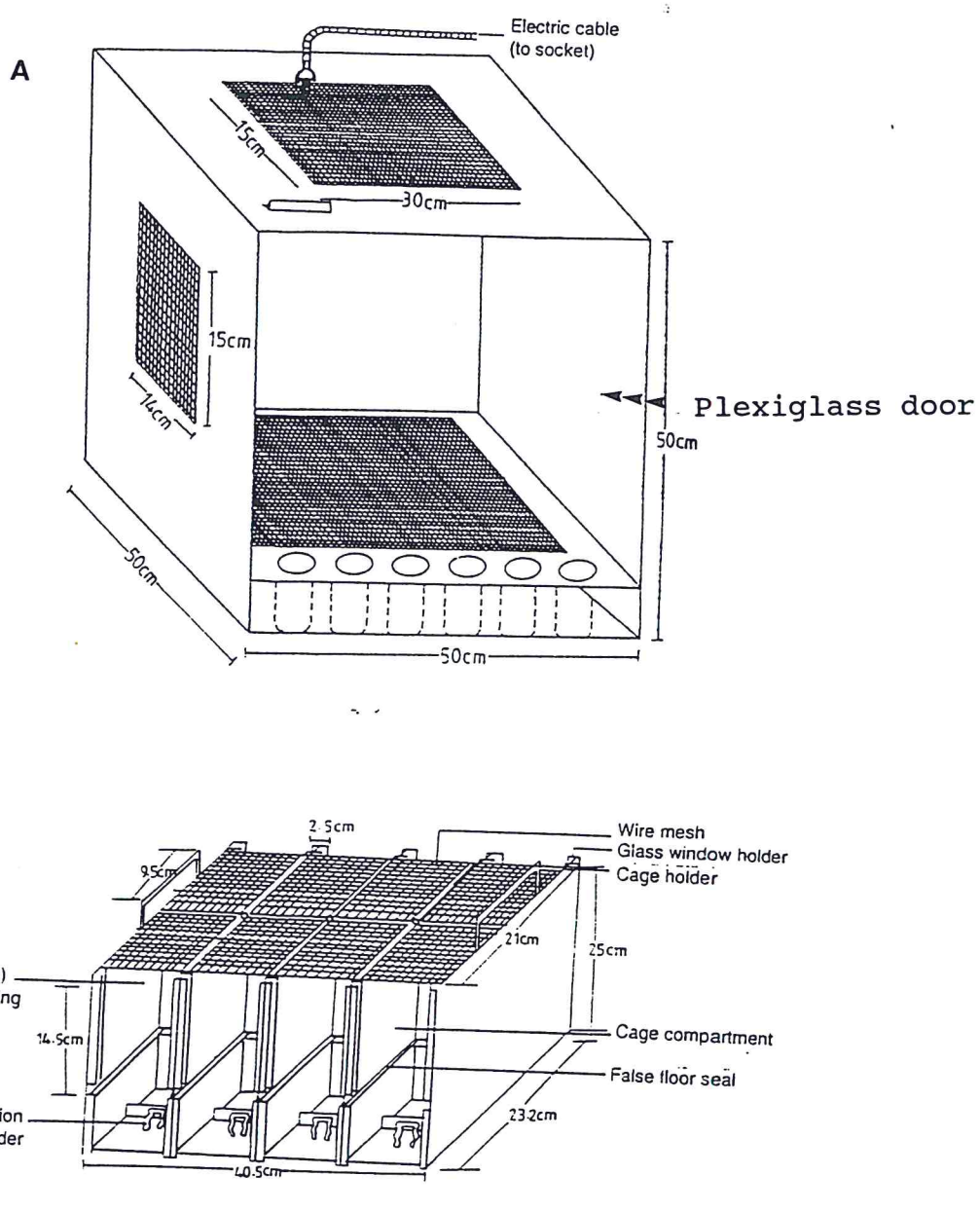


Figure 3. Diagrams of standard cages for rearing crowded (A) and isolated locusts (B)

flow of fresh air at 10-15 air changes per hour (Ochieng-Odero et al., 1991).

Fresh shoots of wheat (variety 'Nyangumi') and wheat bran obtained from Nakuru (Kenya) were provided daily to the colony.

3.2 Bioassays

3.2.1 Single chamber olfactometer

The olfactometer (Fig. 4) was a 60 x 30 x 30 cm single glass chamber with a two choice arena whose top was covered with a removable wire gauze (Obeng-Ofori et al., 1993). An aluminium metal plate drilled with 2 mm-diameter holes (1cm apart) was fitted at the bottom of the chamber. Attached to each half of the arena and from underneath was a 28 cm (base length) pyramidal aluminium funnel. Each of the two funnels was connected by teflon tubing to a 2-litre round bottomed flask. Insects were placed in one of the flasks to serve as the source of volatiles; the other flask was left empty to act as a control. The olfactometer was placed under an extraction hood which maintained a continuous flow of air through the chamber, and prevented accumulation of volatiles. Two 60 cm fluorescent light tubes, each 60 W, were fitted

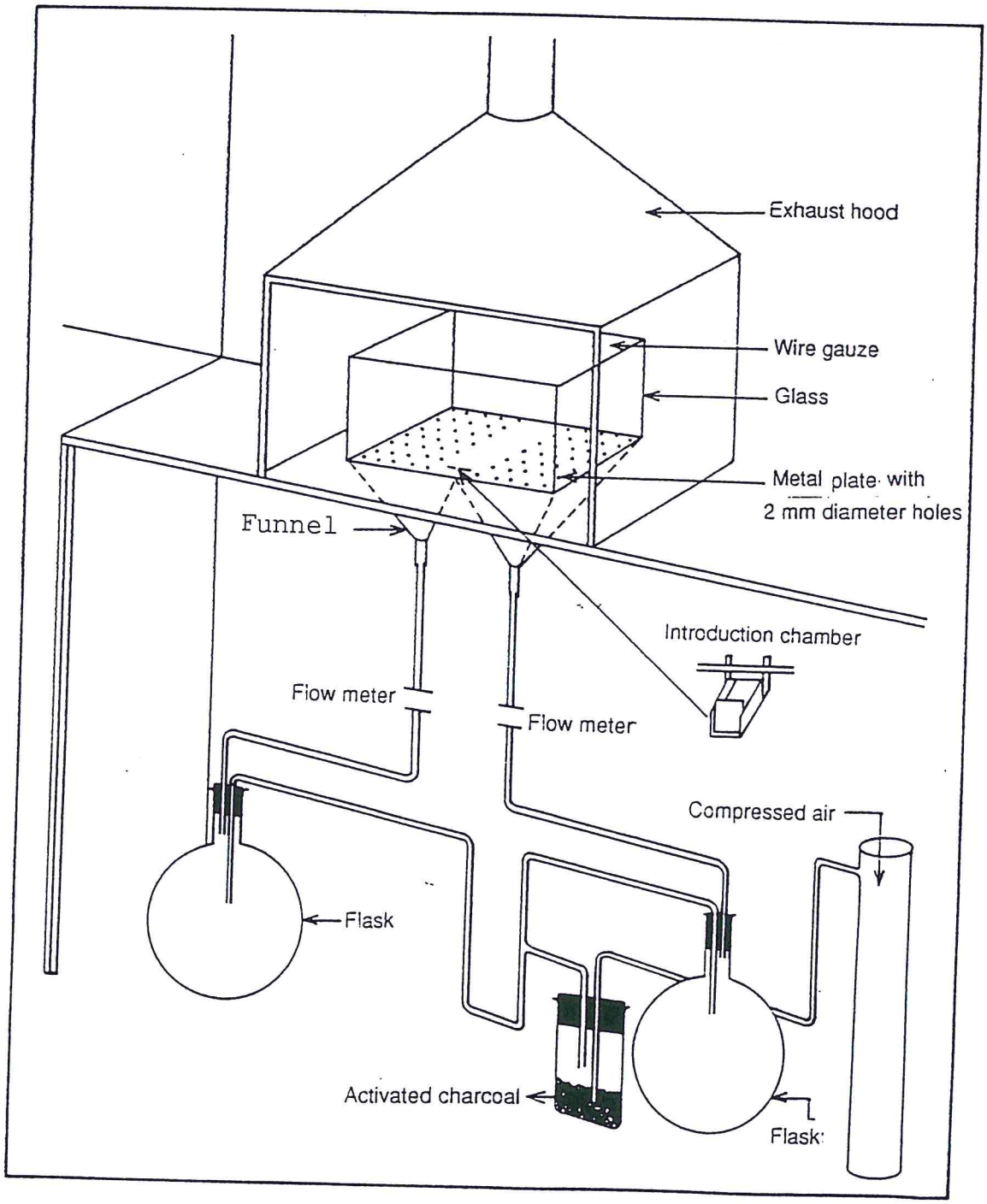


Figure 4. Diagram of the olfactometric aggregation bioassay system

over the olfactometer arena to provide uniform illumination.

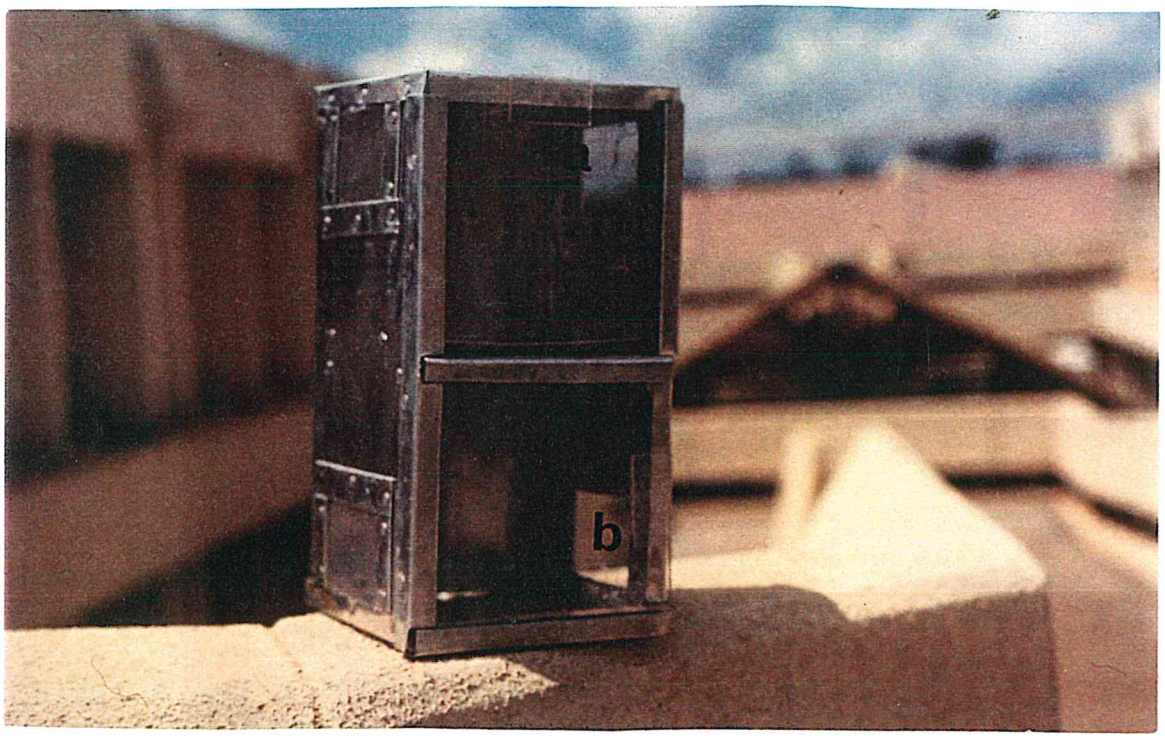
Temperature in the experimental room was maintained at 26-29°C.

3.2.2 Cage assays

Bioassays to investigate the gregarization of solitarious desert locusts in the presence of gregarious colony of migratory locusts were conducted in standard 50 x 50 x 50 cm aluminium cages (Ochieng-Odero, 1991) which were easy to clean and had no risk of adsorbing volatiles (Fig. 3A). The front side of the cage had a sliding plexiglass door to allow observations on the locusts. One of the lateral sides had an opening with a sliding door to enable placement of food. The floor had a wiremesh (5 mm mesh) which allowed the faeces to drop out of the cages. The large cages had an outlet in the back of their ceiling for a 40 W bulb to provide light and heat to the insects inside.

For bioassays where only the effects of volatiles on phase shift or maturation were tested, double storey aluminium cages (15 x 15 x 30 cm), described by Mahamat et al. (1993) were used (Plate A). Wire gauze partition

A



A. Two chamber bioassay cages. a) source chamber, b) recipient (test) chamber.

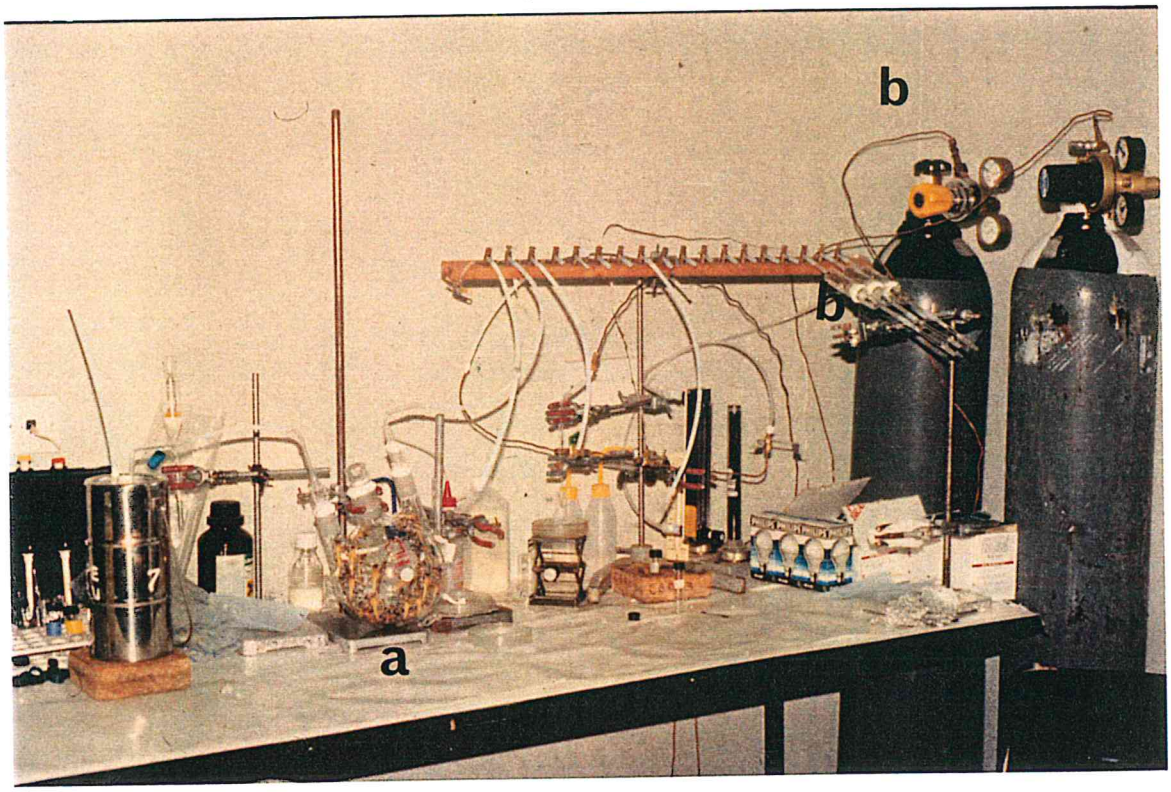
between the two chambers allowed flow of volatiles from the top chamber to the lower one.

3.3 Collection of volatiles

Volatiles were adsorbed onto charcoal traps employing the method described by Torto et al.(1994) (Plate B-1). The traps were 8mm-diameter and 8cm long glass tubes inside which, 60 mg of activated charcoal (80 - 100 mesh Chrompack, Middlesburg, The Netherlands) was packed between two glass wool plugs (Plate B-2d). Before use, the charcoal was cleaned thoroughly by Soxhlet extraction with dichloromethane (Merck, Germany) for 48 hours followed by activation under nitrogen (250 ml/min) at 250°C for one hour. Air from a compressed air cylinder was cleaned by passing it through a charcoal filter before passing it over locusts contained in 0.5, 1.0, or 2 l round-bottomed flask (Plate B-1a) depending on the number of locusts. When trapping was from smaller numbers of locusts (1 - 2 adults or 30 - 50 early instar nymphs), quick-fit glass tubes (male 2.8 cm ID, female 3.4 cm ID, and length 9 cm) (Plate B-1b; B-2c) were used. All joints were sealed with teflon tape to avoid air leakage during trapping sessions. Each trapping session lasted overnight (16 hours) after which, traps were

B

1



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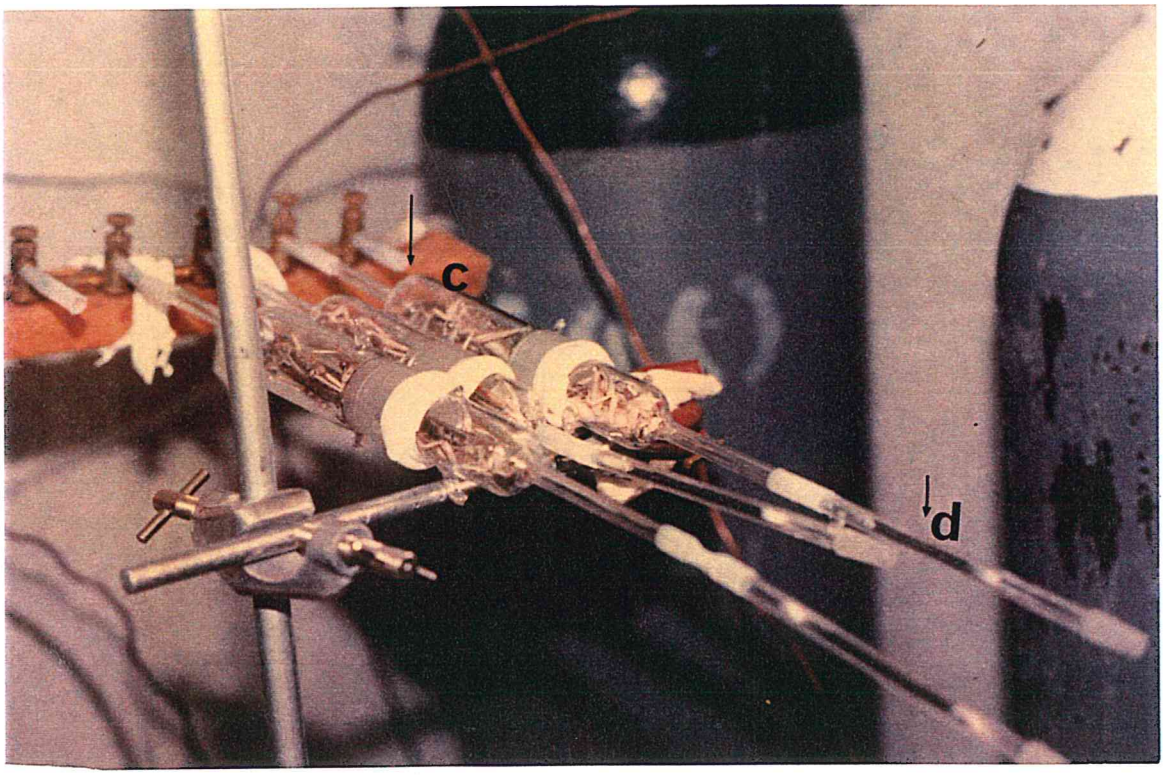


B. Volatile collection system (B-1) showing connected to it a) three-neck round bottomed flask containing mature *S. gregaria* b) quick-fit trapping chambers, and the medical air source cylinders; quickfit chambers (c) with connected charcoal traps (d) (B-2)

eluted into a vial placed in ice with 5 ml HPLC grade (Aldrich Ltd, UK) dichloromethane. Volatile extracts were stored at -15°C, and concentrated under a stream of nitrogen at 0°C to approximately 300 μl prior to analysis.

3.4 Analyses of volatiles

Analyses of volatiles were carried out by capillary gas chromatography (GC) on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a HP capillary column methyl silicone SPB-1 (30m x 0.2 mm ID x 0.2 μm film thickness), or Carbowax (50m x 0.2 mm ID x 0.2 μm film thickness). When the Carbowax column was used, the analyses were run at initial temperature of 60°C isothermal for 10 min, then programmed to 180°C at a rate of 5°C/min and maintained there for 5 min, then to 220°C at a rate of 10°C/min and maintained isothermal for 15 min. When the methyl silicone column was used, the analyses were run at an initial temperature of 45°C for 5 min, then programmed to 150°C at the rate 5°C/min, then to 250°C at a rate of 10°C/ min and maintained there for 13 min. Chromatographic peaks were integrated using a HP 3396 Series II integrator. Samples from *L. migratoria* were analyzed by GC-MS on a VG 12-250 mass spectrometer (EI, 70 eV) coupled to a HP5790 gas chromatograph. The

GC conditions were the same as for analyses using Carbowax.

3.5 Integumental colour grading

Test locusts, *S. gregaria* were monitored for visible colour changes of the cuticle as an indication of gregarization (Plate C-E) or sexual maturity, in accordance with the colour classification of Norris (1954) for adult locusts as follows :

I : insect totally deep pink ;

II : insect clear pink;

III : insect with slight pink and yellow on the abdomen, thorax, hindwings, and frons. This is obvious in male *Schistocerca* and *Locusta* (frons);

IV : Yellow colour generalized over body and hindwings;

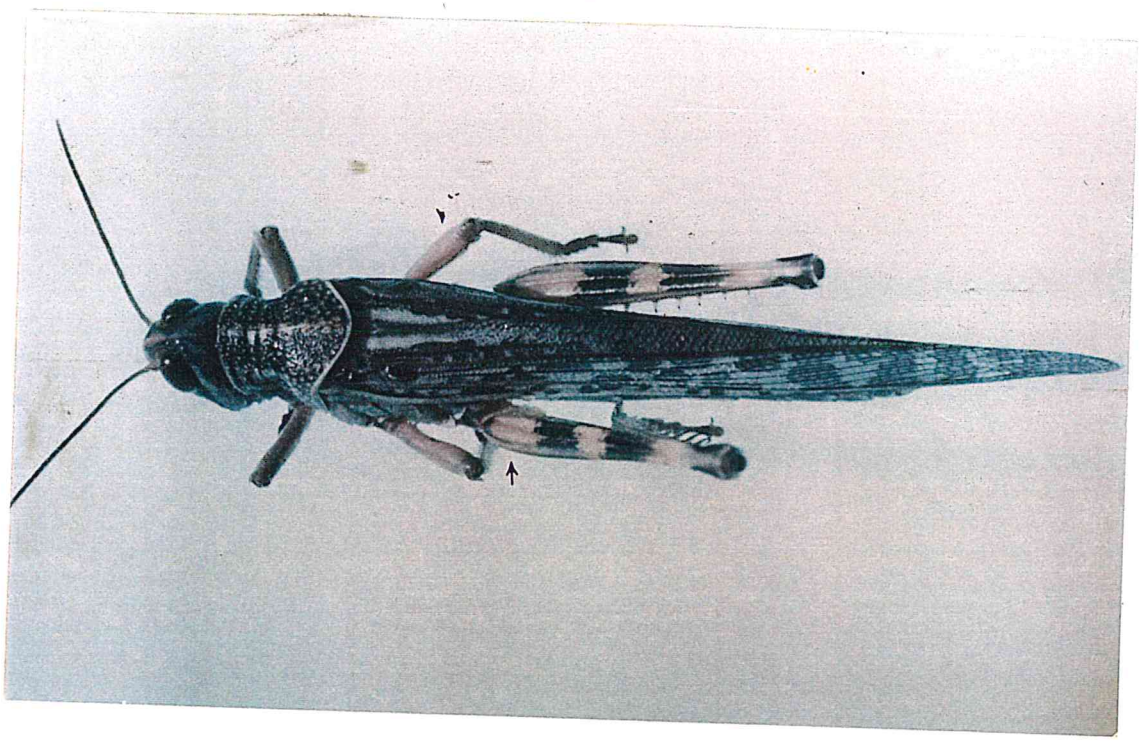
V : Insect totally yellow even females showing frons and hindwings yellow;

Pink immature adults (Plate C-1) or stage III yellow mature adults (Plate D-1) were considered to have reached the transient or gregarious phase.

Immature gregarious adult *L. m. migratorioides* (Plate C-2), also show some faint pink colour but usually pale to creamy white frons. The hind wings are hyaline at soft immature adult stage but become yellow after

C

1



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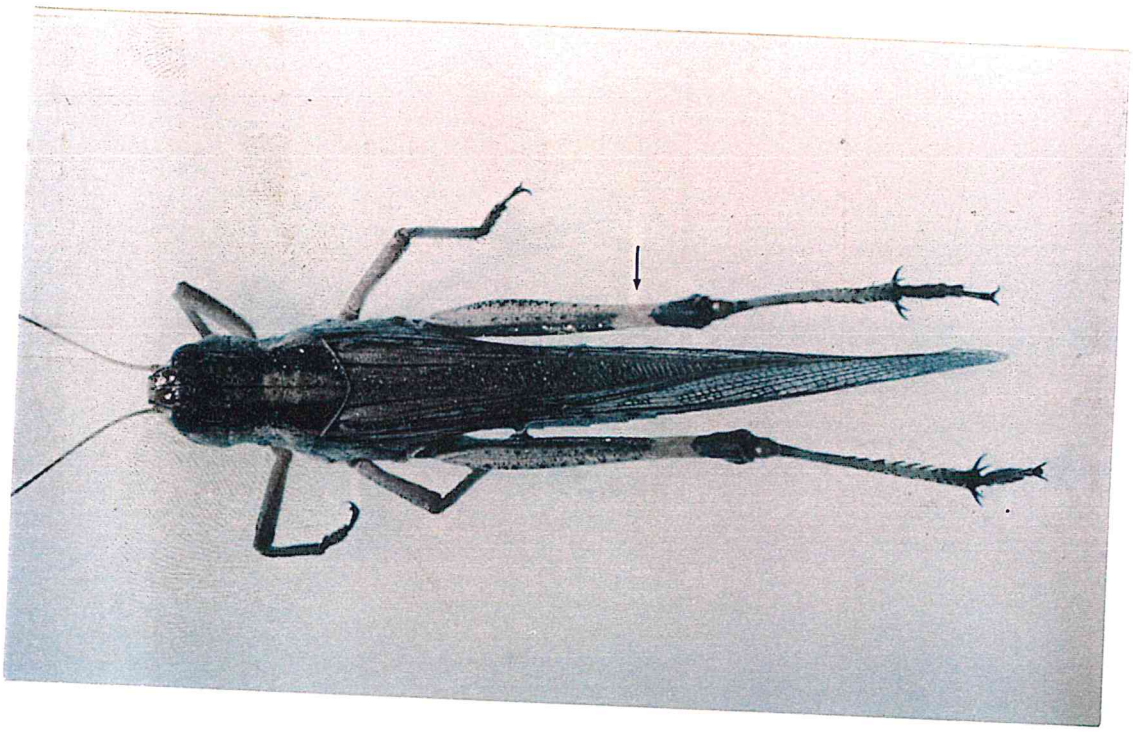

C. Immature adults of *S. gregaria*(C-1) and *L. migratoria*(C-2)(note the pink and blackish body colours of the respective insects).....62

D

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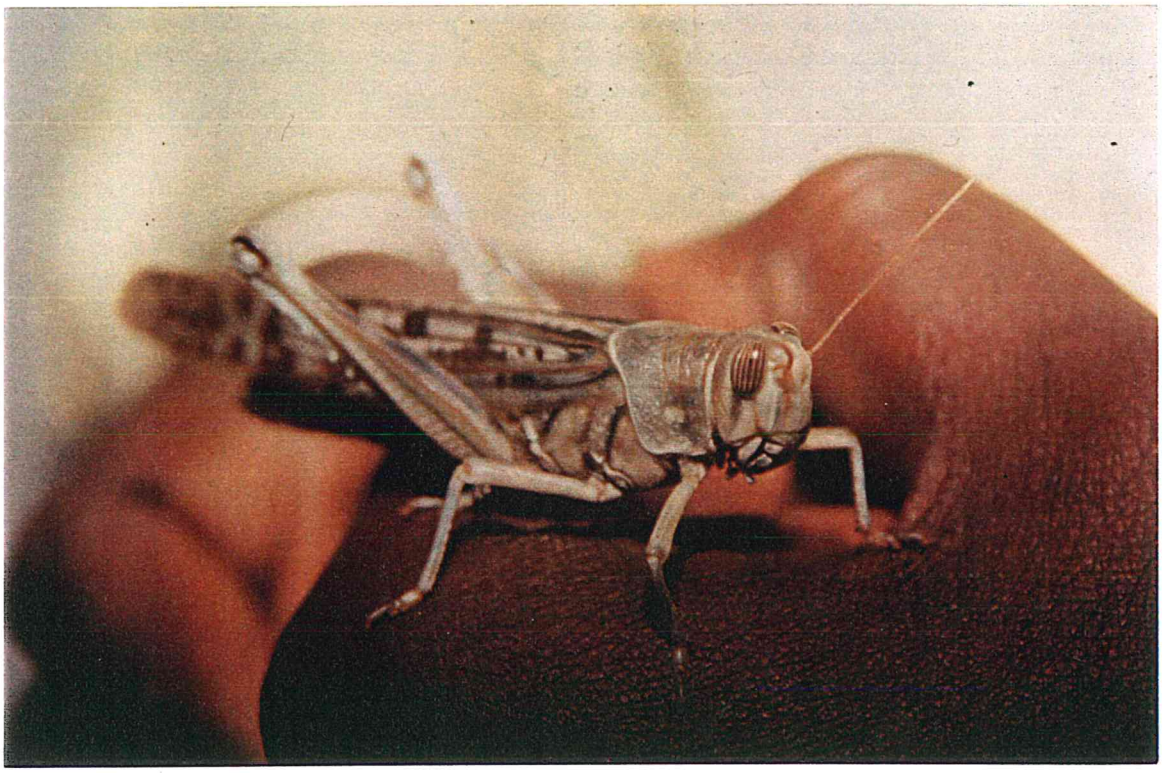
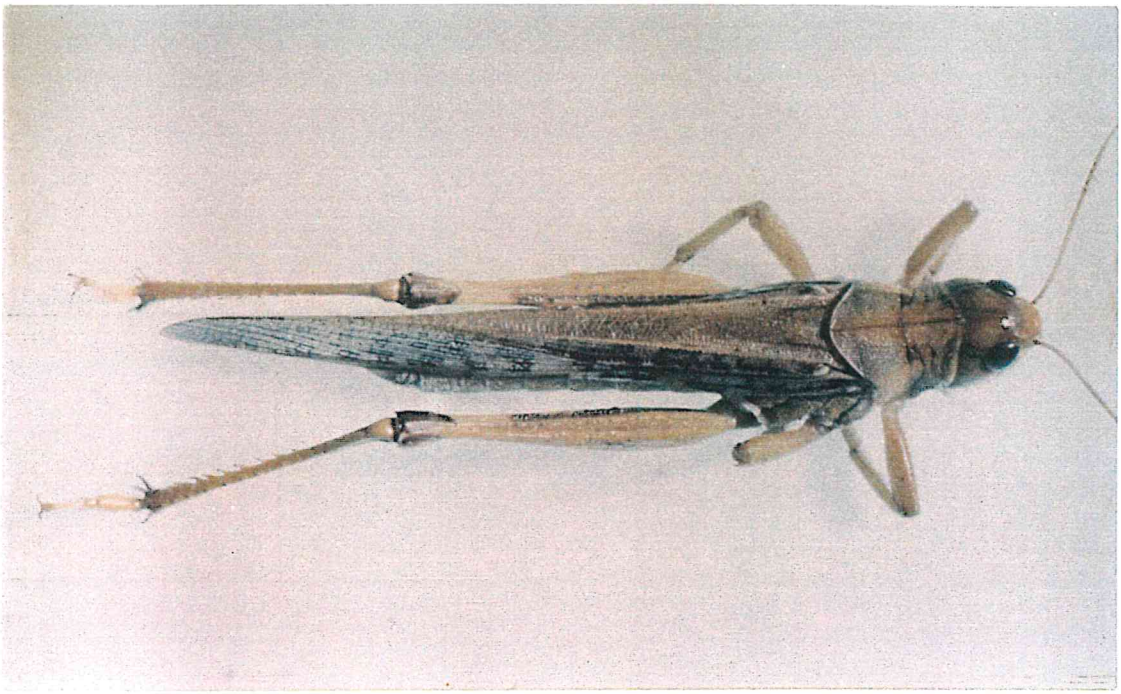


D. Gregarious (D-1) and solitarious (D-2) mature adults of *S. gregaria*.

E

1



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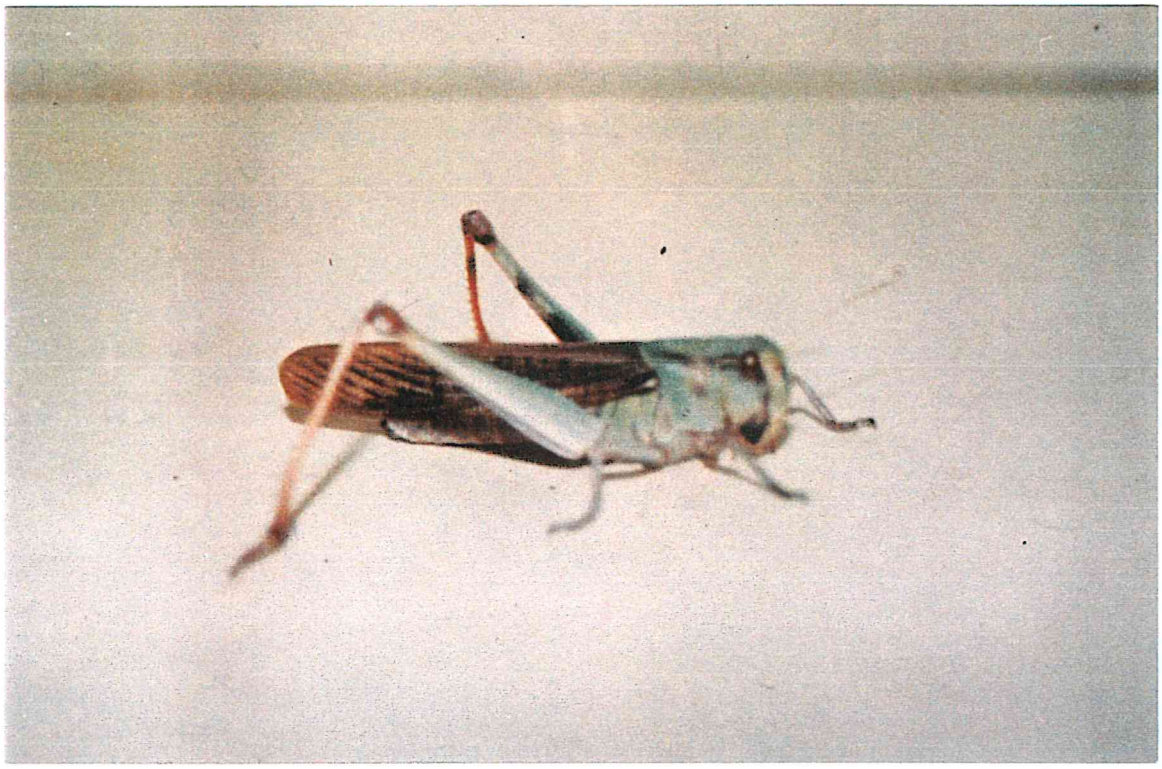
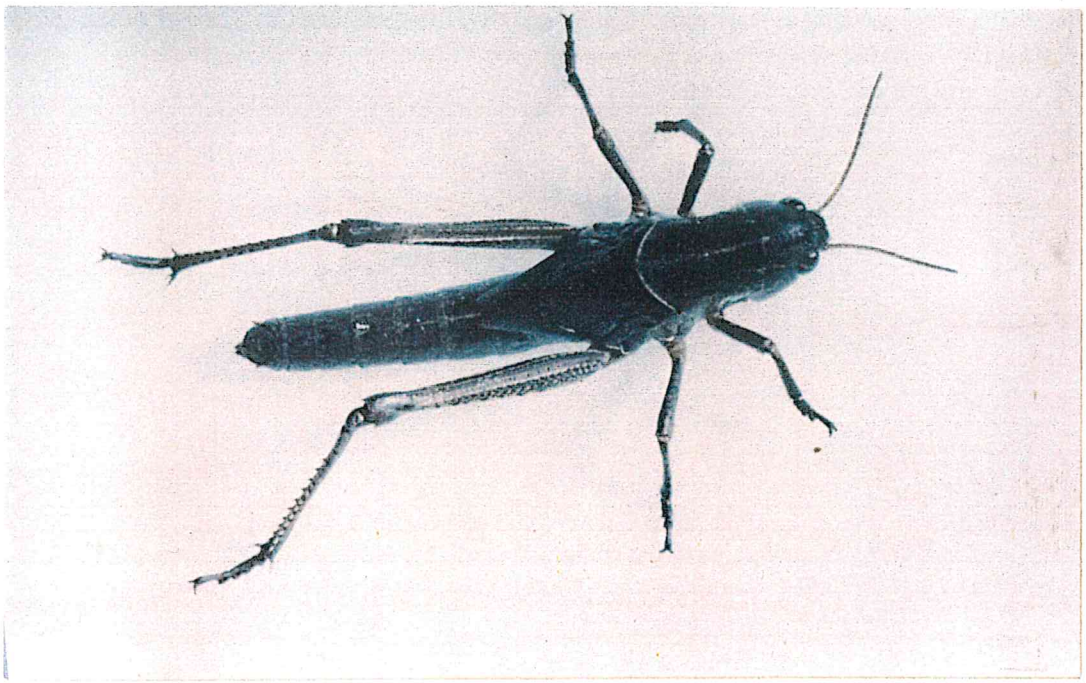


E. Gregarious (E-1) and solitarious (E-2) adults of *L. migratoria migratorioides*

F.

1



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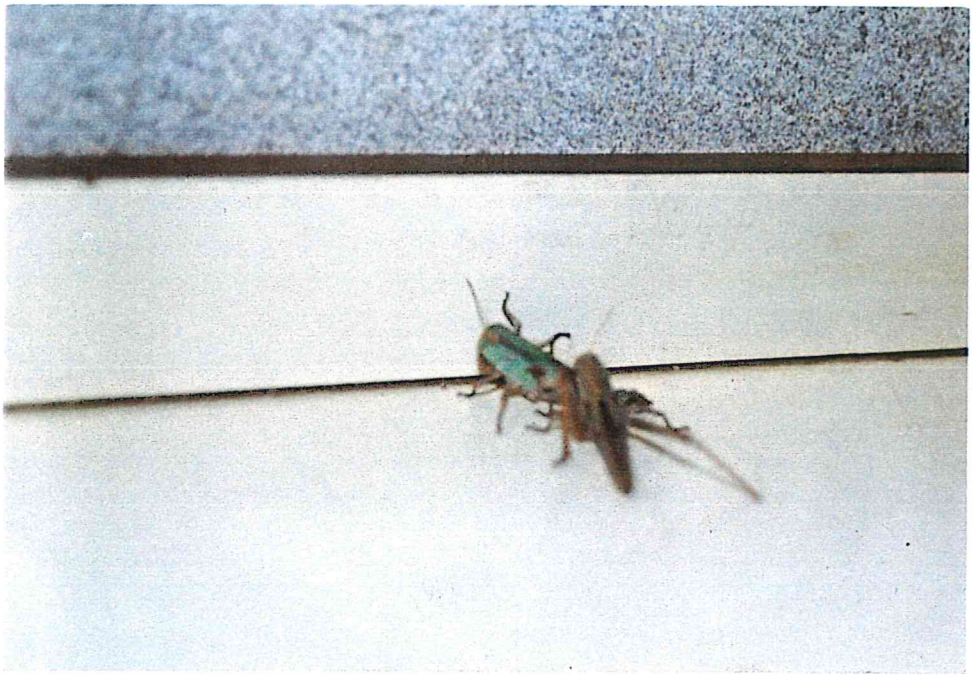


F. Gregarious fifth-instar (F-1) and solitarious fourth-instar (F-2) nymphs of *L. migratoria migratorioides*.

cuticular hardening. The frons turns progressively yellow, especially in males, followed by the pronotum and legs as the locust matures. Solitarious adults (Plate E-2) have a green pronotum head and legs while the hind wings' hyaline colour turns yellow as the insect matures. The front wings are golden greyish. Mature solitarious males although green in general appearance can show yellow frons at maturity. Thus, in the adult migratory locust the yellow colouration on frons was a good indicator of sexual maturity. The colour grading was limited to the extent of the yellow colouration on frons of both gregarious and solitarious adult males, and the green colouration on the pronotum and legs for both male and female solitarious adults. The following scale was adopted:

Grade 1 : No yellow apparent, frons pale with the brown colour fading out. Hind femora with no yellow;

Grade 2 : Frons are pale with some faint yellow, less than 20% appearing at the base of the frons. Hind femora not yellow;

Grade 3 : About 50% of frons yellow with some yellow appearing on the pronotum and hind femora;

Grade 4 : More than 50 % of frons yellow and hind femora with more pronounced yellow;

Grade 5 : Almost whole frons yellow, hind legs and pronotum with more pronounced yellow colouration.

Grade 3 colouration corresponded to sexual maturation of the locusts as males stridulated,

copulated, and produced pheromone whereas adult females started ovipositing.

3.6 Statistical analyses

Statistical analyses were carried out by SAS General Linear Model Procedure (GLM) in which simple or multiple analysis of variance (ANOVA, MANOVA) were run, with the Least Significant Difference test (LSD test at $P \leq 0.05$) for mean comparisons (SAS, 1988). Counts in the olfactometer, morphometric ratios, and haemolymph absorbance ratios, were transformed into arcsine, square roots, log(x), or log(x+1), prior to analysis.

CHAPTER FOUR

4 Aggregation responses of *S. gregaria* and *L. migratoria migratorioides* to their airborne volatiles.

4.1 Introduction

Fuzeau-Braesh et al. (1988) identified guaiacol, phenol, and veratrole as three of the four major volatile constituents in the air surrounding fifth-instar nymphs and older adults of *S. gregaria*, and *L. m. migratorioides*. However, they did not study the responses of the species to the crude volatile blend of the other. A more detailed study on the aggregation responses of different stages of *S. gregaria* has been recently carried out by Obeng-Ofori et al. (1993). They found that, aggregation in the desert locust *S. gregaria* is mediated by two sets of releaser pheromones: a juvenile aggregation pheromone produced by nymphal stages to which only nymphs respond, and an adult aggregation pheromone which is specific to adult stages only. Aggregation responses in *L. m. migratorioides* are unknown and neither are cross-aggregation responses between the two locust species. In this chapter, comparative responses of the two locust species to their own and to each other's volatiles are described. In *L. m.*

migratorioides, it was further investigated whether there are stage and sex differences as reported for *S. gregaria* (Obeng-Ofori et al., 1994a)

4.2 Materials and Methods

4.2.1 Olfactometer assays

Aggregation responses of *S. gregaria* and *L. m. migratorioides* to air-borne volatiles were conducted in olfactometric bioassays using the single chamber olfactometer (described in Chapter III, page 51 of this thesis), which eliminates visual and contact stimuli between source and test locusts.

Air from a compressed air cylinder was cleaned by passing it through a charcoal filter, then split into two streams, each passing into a round-bottomed flask, and then into either of the sides of the arena at a flow rate of 120 ml/min. Locusts placed in one of the flasks provided volatiles for the assays. Sets of five, each of mixed or separate sexes of 3-4 day old fifth-instar nymphs, immature, and 25-40 days old mature adults of *L. m. migratorioides* and *S. gregaria* were tested against their own, and to each other's volatiles at five doses 3, 7, 10, 20, and 40 Locust equivalents (LEQ). One LEQ represents the emission of 1 locust from 2L flask for 10

min. The standard dose in this work was set at 10 LEQ, and the dose responses were studied in the range from 0 to 10, and from 10 to 50. Thus in the lower range, doses 7 and 3 (decrease by 3 units) were tested while in the upper range, doses 20 and 40 (increase by a factor of 2) were so. Test insects were introduced into the olfactometer via the entry box, and after 10 minutes, the number of insects on each side of the arena was recorded (Plate G). The aggregation index (AI) for each test was calculated using the equation:

$$AI = 100 (T - C) / N \quad ; \text{ where}$$

T= number of locusts found on the treated side, C= number of locusts found on the control side, and N= total number of locusts tested.

Experiments involved different stages of *S. gregaria* and *L. m. migratorioides (L. migratoria)* as mixed (M) and separate (S) sexes and the controls (*) in the following combinations:

EXPT	SOURCE	RECIPIENT	(SEX)
	Fifth-instar	**Fifth-instar**	
1	*L. migratoria*	*L. migratoria*	(M, S)*
2	*S. gregaria*	*S. gregaria*	(M)*
3	*L. migratoria*	*S. gregaria*	(M)
4	*S. gregaria*	*L. migratoria*	(M)
	Mature adults	**Mature adults**	
5	*L. migratoria*	*L. migratoria*	(M, S)*
6	*S. gregaria*	*S. gregaria*	(M)*
7	*L. migratoria*	*S. gregaria*	(M)
8	*S. gregaria*	*L. migratoria*	(M)
	Fifth-instar	**Immature adult**	
9	*L. migratoria*	*L. migratoria*	(M)*
10	*S. gregaria*	*S. gregaria*	(M)*
11	*L. migratoria*	*S. gregaria*	(M)
12	*S. gregaria*	*L. migratoria*	(M)
	Mature adults	**Immature adults**	
13	*L. migratoria*	*L. migratoria*	(M)*
14	*S. gregaria*	*S. gregaria*	(M)*
15	*L. migratoria*	*S. gregaria*	(M)
16	*S. gregaria*	*L. migratoria*	(M)

Between experiments, the olfactometer was carefully cleaned with acetone. Air was also flushed through the tubes for 2-3 hours to remove any volatile residues. The source and the control flasks were systematically switched in between experiments to minimize bias.

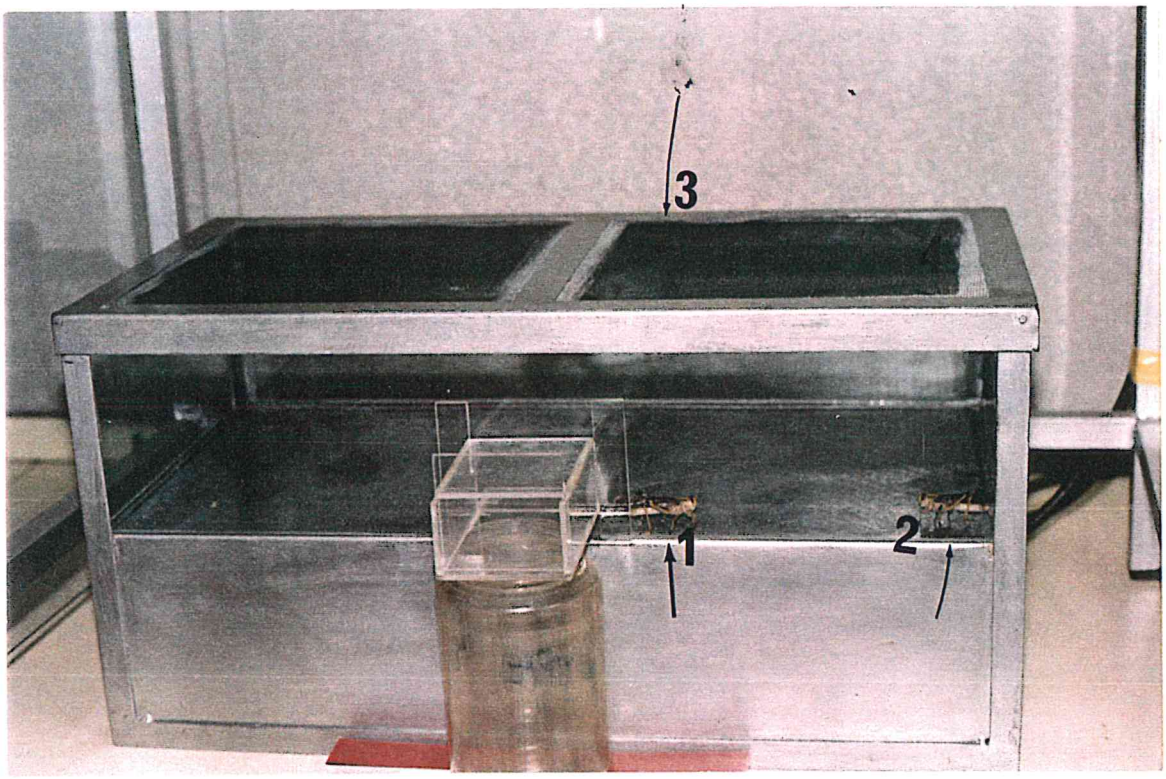
Combinations 1 to 8 were also repeated and distribution of locusts to their volatiles after 10 and 30 min of exposure at the standard dose of 10 LEQ was noted.

Additional tests were conducted with *L. migratoria* for stage and sex-specific responses to volatiles. These involved combinations 1, 5, 9, and 13 in the treatment combination list. Cross responses to eight to ten grams of 80% wet faeces of nymphs and immature adults of the respective locust species were also studied.

4.3 Data analysis

The data were analyzed using the SAS (1988) package. Tests for independence were run using the Chi-square test. For separation of means by treatment, the data were transformed into arcsine or square roots prior to analysis of variance using LSD test at $P \leq 0.05$.

G



G. Olfactometric tests showing locusts (1,2,..) orienting themselves towards or sitting on the treated side of the arena

4.4 Results

4.4.1 Responses of *S. gregaria* to volatiles of *L. migratoria migratorioides*

Aggregation reponses of fifth-instar *S. gregaria* nymphs to their own volatiles and to those of fifth-instar *L. migratoria* nymphs are shown in Fig. 5. Responses were dose-dependent and fifth-instar *S. gregaria* nymphs responded maximally to volatiles produced by *S. gregaria* and *L. migratoria* at 10 and 20 LEQ, respectively. Fifth-instar *S. gregaria* nymphs had similar aggregation responses to their own volatiles and to those produced by fifth-instar *L. migratoria* nymphs. They also responded to faecal volatiles of fifth-instar nymphs of the latter species (Table 2).

Immature adults of the two species had significant aggregation responses to conspecific volatiles. *S. gregaria* immature adults were indifferent to volatiles from nymphs of *L. migratoria* while immature adults of *L. migratoria* were actually repelled by volatiles from conspecific nymphs and responded weakly and insignificantly to those of nymphal *S. gregaria*. On the other hand, immature adults of both species responded equally to volatiles produced by mature adults of the two species, but significantly more to those from their

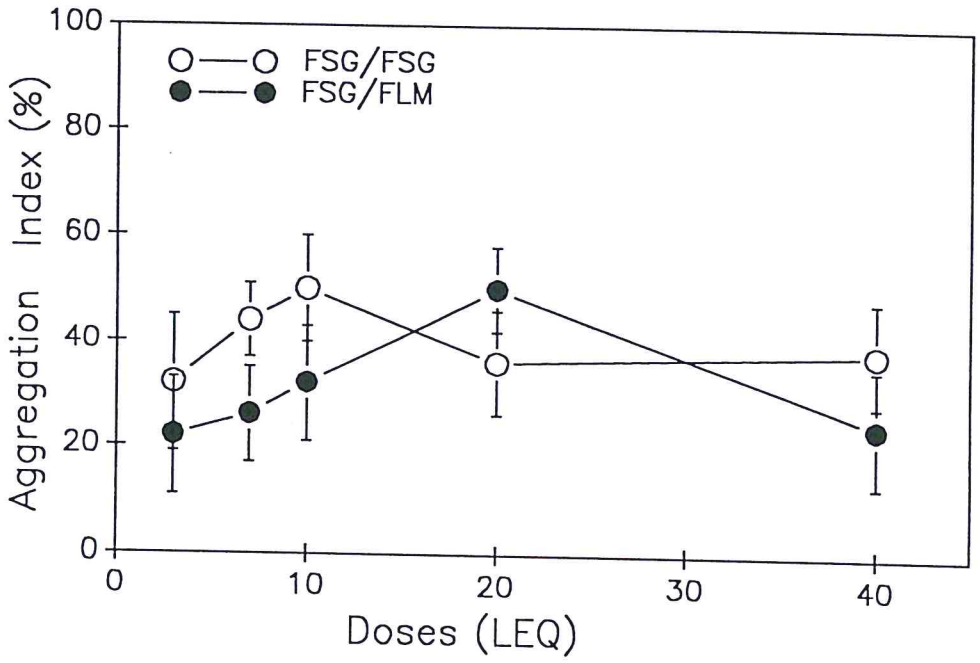


Figure 5. Dose-aggregation response curves for fifth-instar nymphs of *S. gregaria* (FSG) to volatiles of fifth-instar conspecifics **[(FSG/FSG)]** and to those of *L. migratoria* (FLM), **[(FSG/FLM)]** in the olfactometer (bars are SE).

Table 2. Aggregation responses of immature adults of *S. gregaria* (SG) and *L. m. migratorioides* (LM) to nymphal and adult conspecific and interspecific volatiles in the olfactometer.

Source	Test	Aggregation Index (% ± SE)
Fifth-instar	Immature adult	
LM	LM	-34 ± 10 c
SG	LM	16 ± 8 a
LM	SG	-2 ± 8 b
SG	SG	18 ± 8 a
Mature adult	Immature adult	
LM	LM	60 ± 11 a
SG	LM	22 ± 12 b
LM	SG	24 ± 11 b
SG	SG	40 ± 6 b
Nymphal faeces	Fifth-instar	
LM	SG	28 ± 8 a
SG	LM	24 ± 7 a
Nymphal faeces	Immature adult	
LM	SG	32 ± 13 a
SG	LM	32 ± 5 a

Mean indices in each group followed by the same letter are not significantly different(at 5% level, LSD-test).

conspecifics (Table 2). They also responded significantly higher than the untreated control ($P < 0.05$), to faecal volatiles of fifth-instar nymphs of *L. migratoria* similar to the responses to conspecific immature adult fecal volatiles (Table 2).

Responses of mature adults of *S. gregaria* to their own volatiles and those of *L. migratoria* were dose-dependent. Adults of *S. gregaria* aggregated strongly ($P< 0.05$) and significantly more to their own volatiles than to those produced by *L. migratoria* especially at doses of 10 and 20 LEQ (Fig. 6).

4.4.2 Responses of *L. migratoria* to volatiles of *S. gregaria*.

The reponses of fifth-instar nymphs of *L. migratoria* to their own volatiles and to those of fifth-instar nymphs of *S. gregaria* are shown in Fig. 7. Responses to volatiles of nymphal *S. gregaria* were not dose-dependent and there were no significant differences in responses of nymphal *L. migratoria* to their own volatiles and to those produced by fifth-instar *S. gregaria* over the range of doses tested ($P <0.05$) (Fig. 7). However with regard to their own volatiles, nymphs of *L. migratoria* had significantly higher ($P <0.05$) aggregation responses to volatiles from conspecifics at 20 LEQ than at other doses. They also

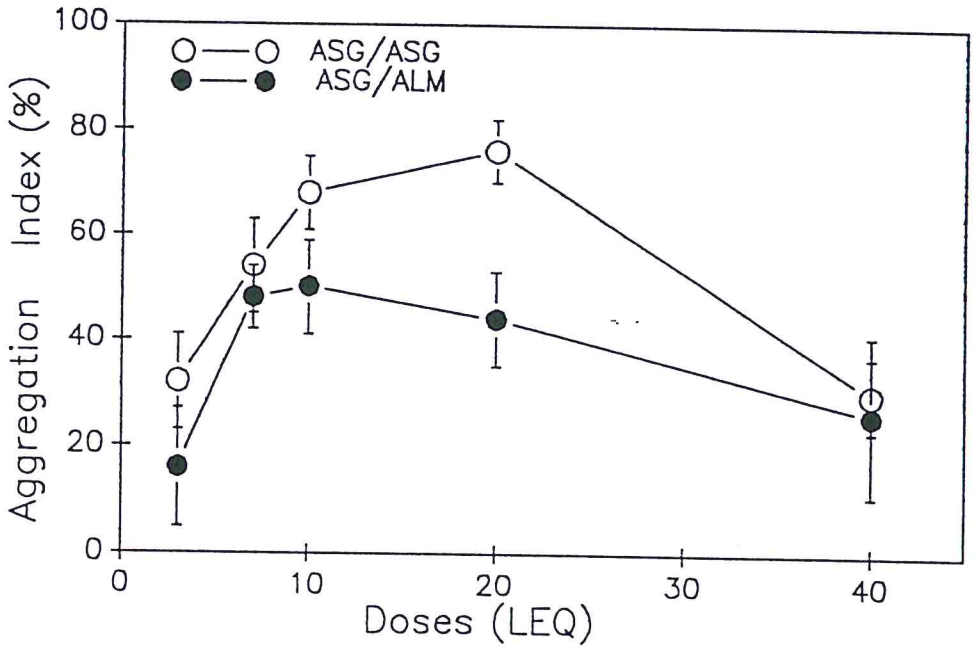


Figure 6. Dose-aggregation response curves for mature adults of *S. gregaria*(ASG) to volatiles of mature adult conspecifics**[(ASG/ASG)]** and to those of *L. migratoria*(ALM), **[(ASG/ALM)]** in the olfactometer(bars are SE).

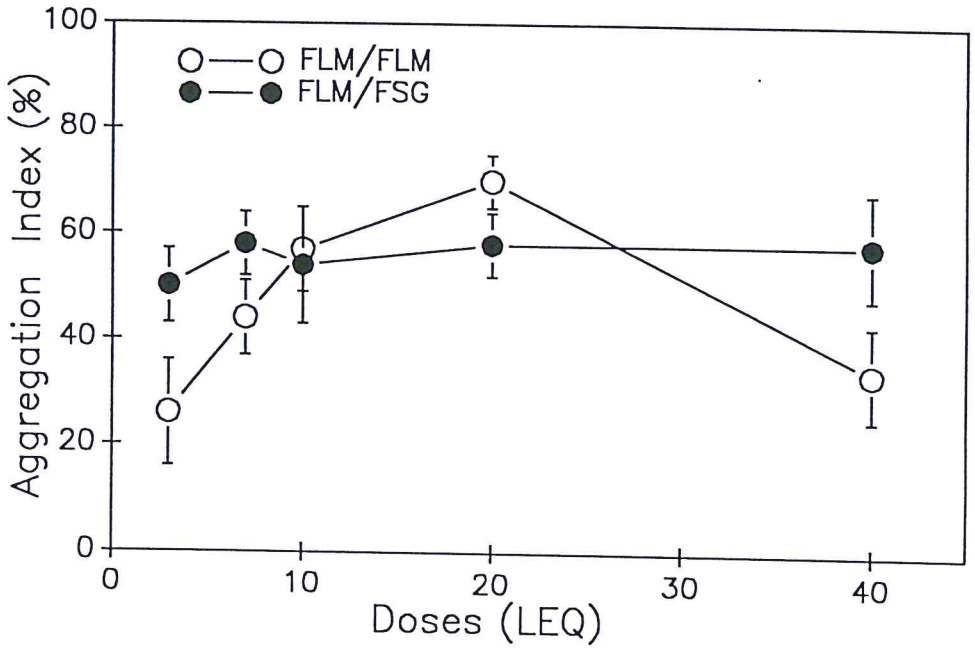


Figure 7. Dose-aggregation response curves for fifth-instar nymphs of *L. migratoria* (FLM) to volatiles of fifth-instar conspecifics **[(FLM/FLM)]** and to those of *S. gregaria*(FSG), **[(FLM/FSG)]** in the olfactometer(bars are SE).

responded significantly (P <.05) compared to untreated control, to faecal volatiles of fifth-instar nymphs of *S. gregaria* (Table 2).

Immature adults of the two species aggregated weakly to volatiles of fifth-instar nymphs. They responded less to volatiles produced by mature adults of *S. gregaria*, compared to responses to their own volatiles. Responses were significantly greater within than between species. They also responded to faecal volatiles of fifth-instar nymphs of *S. gregaria* (Table 2).

Responses of mature adults of *L. migratoria* to their own volatiles and to those of *S. gregaria* were dose-dependent and there were no significant differences over the range of doses tested (Fig. 8).

4.4.3 Responses of *S. gregaria* or *L. migratoria* to choices of volatiles of both species

Nymphs of *S. gregaria* responded to volatiles of nymphs of *L. migratoria* similar to their own. Similarly, there were no significant differences (P > 0.05)in aggregation responses of nymphs of *L. migratoria* to volatiles of *S. gregaria* and to those of their conspecifics during 10 or 30 min exposure (Table 3).

Mature adults of *L. migratoria* showed a significantly stronger response to their own volatiles

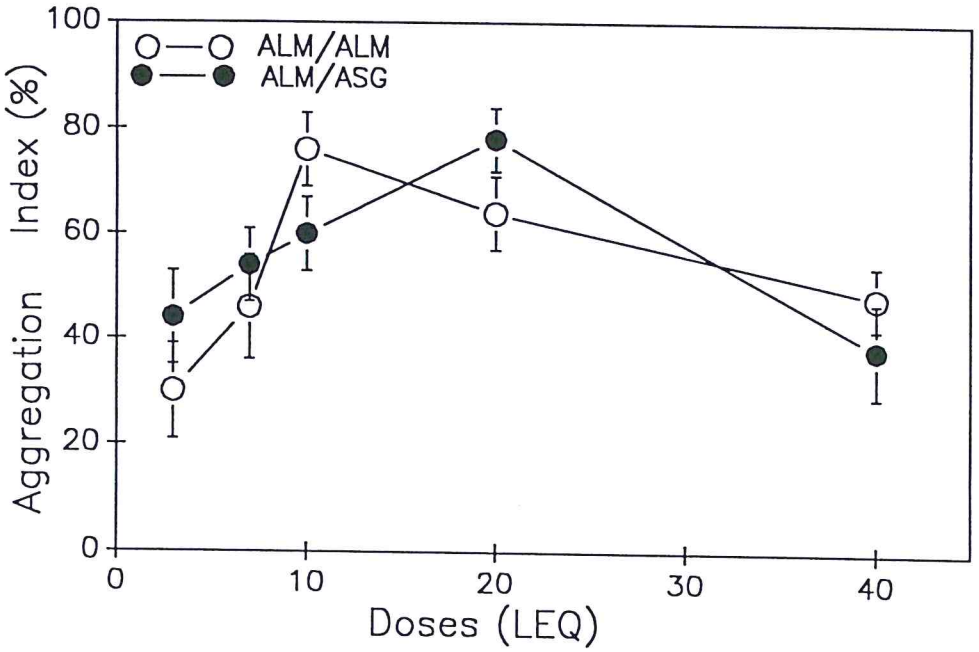


Figure 8. Dose-aggregation response curves for mature adults of *L. migratoria*(ALM) to volatiles of mature adult conspecifics**[(ALM/ALM)]** and to those of *S. gregaria*(ASG), **[(ALM/ASG)]** in the olfactometer (bars are SE).

than to those of *S. gregaria* after 10 min of exposure ($P \leq 0.05$). However, after 30 min, mature adults of *L. migratoria* responded significantly more to volatiles of *S. gregaria* than to their own ($P \leq 0.05$). Mature adults of *S. gregaria* responded to volatiles of mature adults of *L. migratoria* similar to their own at the two time (10 and 30 min) intervals. Aggregation responses to volatiles of *S. gregaria* of the two locust species after 30 min of exposure to volatiles were not significantly different ($P > 0.05$)(Table 3).

4.4.4 Sex responses to volatiles in *L.m. migratorioides*.

Male and female nymphs of *L. migratoria migratorioides* did not show any preference for their respective volatiles (Table 4). Mature adult females were more strongly attracted ($P< 0.05$) to volatiles from mature males than males to those of females. However, mature females showed significantly ($P \leq 0.05$) lower aggregation responses to their own volatiles compared to the aggregation of males to their own, and this was not significantly different ($P > 0.05$) from the control (Table 4).

Table 3. Responses of *S. gregaria* (SG) and *L. m. migratorioides* (LM) to volatiles emanating simultaneously from 10 locusts of each species placed in olfactometer choice tests.

Test (stages)	Time (min)	Aggregation index (% ±SE) to LM volatiles	Aggregation index (% ±SE) to SG volatiles
Fifth-instar			
LM	10	42 ± 6 a	52 ± 7 a
SG	10	42 ± 9 a	60 ± 8 a
LM	30	44 ± 9 a	50 ± 12 a
SG	30	46 ± 6 a	54 ± 6 a
Mature adult			
LM	10	56 ± 8 a	32 ± 5 b
SG	10	44 ± 4 a	54 ± 4 a
LM	30	32 ± 5 b	66 ± 5 a
SG	30	44 ± 8 a	56 ± 9 a

All means in each column(3 and 4), followed by the same letter are not significantly different (at 5% level, LSD test).

Table 4 **Sex responses of nymphs and mature adults of *L. m. migratorioides* to volatiles in the olfactometer.**

Source	Test	Aggregation Index (% ± SE)
Fifth-instar	**Fifth-instar**	
Males	Females	40 ± 9 a
Males	Males	42 ± 11 a
Females	Males	42 ± 9 a
Females	Females	46 ± 13 a
Mature adult	**Mature adult**	
Males	Females	56 ± 6 a
Males	Males	40 ± 8 b
Females	Males	38 ± 9 b
Females	Females	18 ± 8 c

Means in last column and by stage, followed by the same letter are not significantly different (at 5% level, LSD test).

4.4.5 Intra and inter-stage aggregation responses in *L. m.migratorioides*.

Male and female fifth-instar nymphs responded highly to their volatiles which produced a repellent effect on immature adults (Table 5). On the other hand mature adults were indifferent to nymphal volatiles, but responded highly to their own volatiles to which fifth-instar, young, and older adults were also responsive (Table 5).

Table 5 Cross-stage aggregation responses of *L. m. migratorioides* in the olfactometer.

Source	Test	Aggregation Index (% ± SE)
Fifth-instars	Fifth-instars	57 ± 8 a
Fifth-instars	Immature adults	-34 ± 10 c
Fifth-instars	Mature adults	6 ± 10 b
Mature adults	Fifth-instars	40 ± 10 b
Mature adults	Immature adults	60 ± 11 ab
Mature adults	Mature adults	76 ± 7 a

In each group, means followed by the same letter in the last column are not significantly different (at 5% level, LSD test).

4.5 Discussion

The present results show some similarity in the aggregation response patterns of different stages of *S. gregaria* and *L.m. migratorioides*. Particularly noteworthy is the extent of cross-reactivity between the nymphal and mature adults stages respectively of the two species. Thus, the aggregation responses of fifth-instar nymphs to their respective volatiles as well as to those of one another were remarkably similar (Figs. 5 and 7). As in the case of *S. gregaria* (Obeng-Ofori et al., 1994a), no sexual differences in the emissions of, or responses to, the nymphal pheromone was observed in *L. migratoria*. Nymphs, and immature adults cross-responded to their fecal volatiles. These results suggest the presence of either similar compounds or pheromone blends with similar releaser effects on each other's nymphs in the nymphal emissions of these species. This may account for the occurrence of mixed hopper bands of *S. gregaria* and *L. m. migratorioides* often observed in the field (Johnston and Buxton, 1949; El-Bashir and Abdel-Rahman, 1991; Torto, pers. comm.). Similarly, mixed populations of immature adults and fifth-instar nymphs could be explained by the fact that immature adults of the two locust species are responsive to each other's nymphal faecal volatiles.

The responses of mature adults to their respective volatiles as well as to those of one another were also quite similar (Figs. 6 and 8). However, in this case, *S. gregaria* responses to volatiles of conspecifics was significantly higher at some doses (10 and 20 LEQ) compared to its responses to volatiles of mature adults of *L. m. migratorioides* (Fig. 6) suggesting some differences in the composition of the two pheromone systems or possibly in the amounts of the active compounds.

On the other hand, significant differences were observed between the two species when the aggregation responses of one stage of the insect to volatiles of another were compared. Thus, although immature adults of *S. gregaria* were indifferent or aggregated weakly to volatiles of conspecific fifth-instar nymphs as previously reported (Obeng-Ofori et al., 1993), those of *L.m. migratorioides* were significantly repelled by conspecific nymphs of similar stage (Table 2). Interestingly, immature adults of *L. m. migratorioides* were stimulated to aggregate, albeit weakly, in response to volatiles of fifth-instar nymphs of *S. gregaria*, whereas *S. gregaria* immature adults were indifferent to volatiles of nymphal *L. m. migratorioides*. These results clearly point toward compositional differences in the nymphal volatile blends of the two species. Comparison

of the aggregation responses of immature adults to volatiles of conspecific and interspecific mature adults also shows compositional differences in the adult pheromone blends (Table 2). Thus, although immature adults of both species aggregated significantly to volatiles of conspecific mature adults, their cross responses were weak and statistically insignificant. Differences in pheromonal responses between the two locusts both at nymphal and adult stages may also be quantitative, inferring then that the two locusts have different treshold responses to the pheromones, and probably produce different amounts of the necessary compounds. This implies that in the ecological point of view these two locusts still maintain their specificity as two different species with distinct niches.

Cross responses between mature adults and nymphal stages also show differences between the two species (Tables 2 and 5). Thus, whereas in *S. gregaria* mature adults and nymphs are indifferent to each other's volatiles, in *L. m. migratorioides*, although mature adults are also indifferent to nymphal volatiles, the nymphs respond positively to those of mature adults.

A major difference between the adults of the two locusts is that in *S. gregaria* males do not respond significantly to volatiles from females (Obeng-Ofori et al., 1994), while in *L. m. migratorioides* both males and

females cross responded to each other's volatiles. In the latter species (Table 4), females were strongly attracted to male volatiles, and, conversely males were significantly strongly attracted to female volatiles. The attraction of females to female volatiles was very weak and statistically insignificant. These results suggest the mediation of two sets of volatile pheromones in mature adults of *L.m. migratorioides*: an aggregation pheromone produced by males that elicits aggregation from conspecifics of both sexes, and a sex pheromone that is produced by mature females that attracts/arrest male conspecifics.

In summary, the present study has demonstrated some similarities as well as differences in the pheromone-mediated aggregation behaviour of *S. gregaria* and *L. migratoria migratorioides*. The nymphs, as illustrated by the fifth stadium, were remarkably similar and indeed, the two species cross-responded to each other's pheromones; but *S. gregaria* showed some specificity for its own pheromone. Major differences between the two species occurred in cross- stage responses and in sex differentiation in the pheromone systems of mature adults. It is concluded that despite cross-activity between the aggregation pheromones of the two species both the nymphal and adult aggregation pheromones have

significantly different compositions possibly with some overlapping compounds. In addition, *L. migratoria* has a volatile sex-recognition signal produced by females.

CHAPTER FIVE

5. Primer effects of a gregarious population of one species on solitary individuals of the other.

5.1 Introduction

Locusts, as well as other living creatures, interact with their environment in general, and particularly with other insects sharing the same habitat. The behaviour and maturation of the desert locust and the migratory locusts have been reported to be affected by the presence of other locusts (Gillett, 1968; Loher, 1990) and host plants(Jackson et al., 1978; Assad, 1995). Nolte (1977), Ba-Angood (1976), and Gillett (1968) showed that when solitarious locusts were reared with certain grasshoppers their morphometrics, colour, and eye stripes did not change, but when reared with other locust species, they acquired some gregarious characteristics.

Studies described in Chapter IV show that there are strong pheromone-mediated interactions between the two locust species. The long term (primer) effects resulting from interactions between the two locust species, *S. gregaria* and *L. migratoria migratorioides*, are the subject of this Chapter. Primer effects were assessed by more sensitive phase markers such as pheromone production

(Deng et al. 1996) and haemolymph pigment composition (Mahamat et al., 1996) in addition to previously known characters based on body colour changes and morphometrics.

5.2 Materials and methods

This section addresses three different objectives. The first one is on the investigation of the effects of gregarious migratory locusts and two grasshopper species on the gregarization of solitarious desert locust in mixed population; the second is on the investigation of the effects of gregarious desert locusts on the gregarization of solitarious migratory locusts; and the third is on the effects of the volatiles of gregarious individuals of one species on the gregarization of solitarious ones of the other, with *S. gregaria* responses to volatiles of *L. migratoria* taken as a case study.

5.2.1 Effects of the presence of gregarious *L.m. migratorioides* and two grasshopper species on the gregarization of solitarious *S. gregaria*

Insects and experiments

Preliminary rearing tests at random densities of 30, 60, 70, 80, 110, 120, 130, 180, 200, 270, 400 nymphs and adults of *L. migratoria migratorioides* were conducted to determine the appropriate locust density to use as stimulus source on *S. gregaria* in subsequent assays. Integumental colour changes were used to monitor the degree of gregarization in test locusts. All locusts tested shifted to gregarious colour (grades 4 and 5, see page 59-52) at all densities tested. A standard density of 80 locusts was used in subsequent assays. The size of the cage (50 x 50 x 50 cm) allowed free movement of the locusts. One solitary desert locust, at nymphal or adult stages, was placed in the cage already described in Chapter III (Plate H) containing specific stages of either 80 two-day-old nymphs of gregarious *L. migratoria*, *Phymateus viridipes* Stål, Pyrgomorphidae, or *Eyprepocnemis plorans* (Charpentier), Eyprepocnemidinae. The latter two are grasshopper species collected as first instars behind the ICIPE fence.

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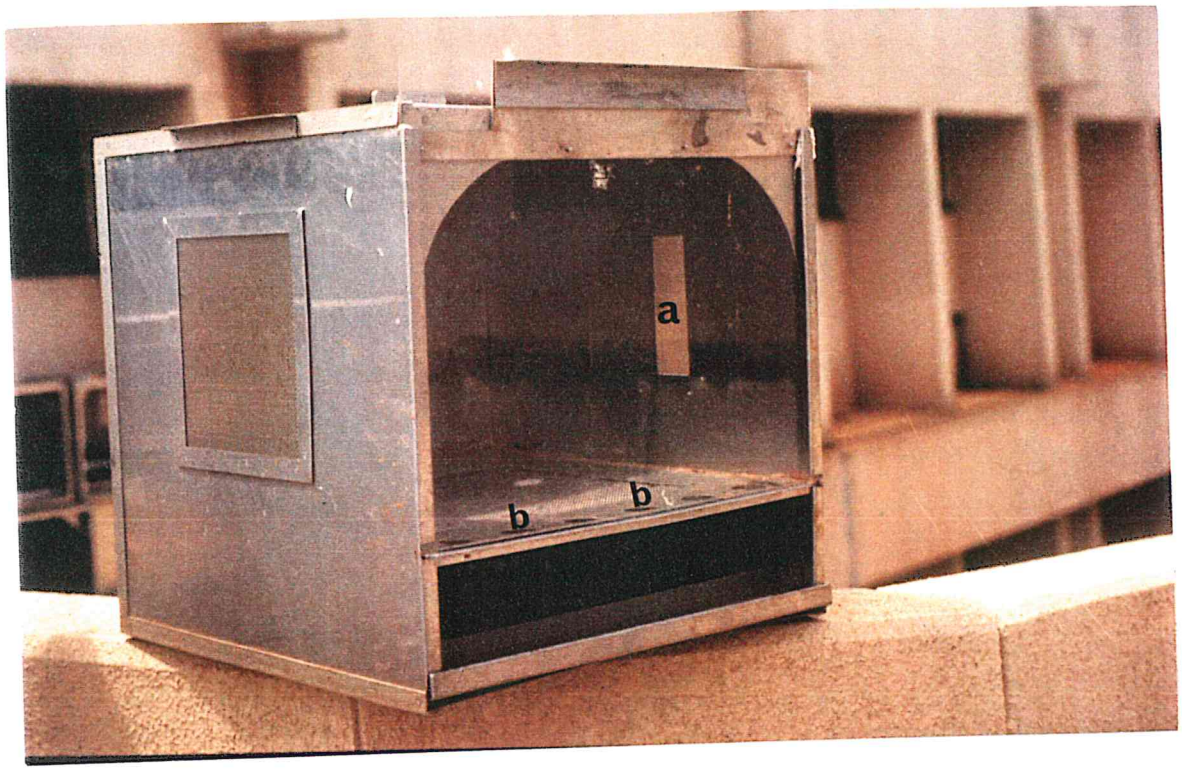

H. One chamber bioassay cage used for mixed rearing experiments and described in Chapter III, and showing a) glass door, b) egg laying tube insertion holes.

Treatments (TRT) were designed as follows:

TRT	STIMULUS	SOLITARIOUS TEST	RATIO	REPS
1.	First-instar *L. migratoria*	First-instar *S. gregaria*	80 : 1	19
2.	Two-day-old immature adult *L. migratoria*	Two-day-old immature adult *S. gregaria*	80 : 1	9
3.	Fifth-instar *L. migratoria*	Two-day-old immature adult *S. gregaria*	80 : 1	10
4.	First-instar *P. viridipes*	First-instar *S. gregaria*	80 : 1	8
5.	First-instar *E. plorans*	First-instar *S. gregaria*	80 : 1	2
6.	First-instar *S. gregaria*	First-instar *S. gregaria*	80 : 1	19
7.	First-instar *S. gregaria*	First-instar *S. gregaria*	0 : 1	19

Treatments 6 and 7 were referred to in the text as control gregarious or solitarious respectively. Test nymphal locusts were monitored for changes in integumental colour, and durations of instars (and stage) while adult locusts were monitored for changes in integumental colour, eye shading, morphometrics (E/F and F/C ratios), eye colour, haemolymph pigment composition, and titres of the male-produced aggregation pheromone.

Integumental colour

Nymphal colour changes were recorded daily, following Stower (1959) nymphal colour grading as follows (Fig. 9):

0 : no black maculation on head, abdomen and legs.

1 : a few black spots on the head; less than 1/4 of the abdomen is black; a black line appearing in the mid horizontal axis of the femur.

2 : 1/3 of head including mouthparts black; less than 1/4 of the abdomen is black; black line on the femur conspicuous.

3 : 1/2 of head and mouthparts black; less than 1/4 of the abdomen is black; femur over 1/3 black.

4 : 2/3 of head and mouthparts are black; 1/4 of the abdomen is black; 1/2 femur is black.

5 : over 2/3 of head and 1/2 of abdomen and femur black (Plate I-1; I-2).

Adult colour changes from immature pink stage to mature yellow stage were also recorded daily until maturation colour stage III was attained as described in Chapter III.

Number of nymphal instars, nymphal and total developmental (to mature adult stage) times.

The nymphal developmental time in the desert locust was determined by monitoring the number of instars. This was done by recording each nymphal moult, and by counting

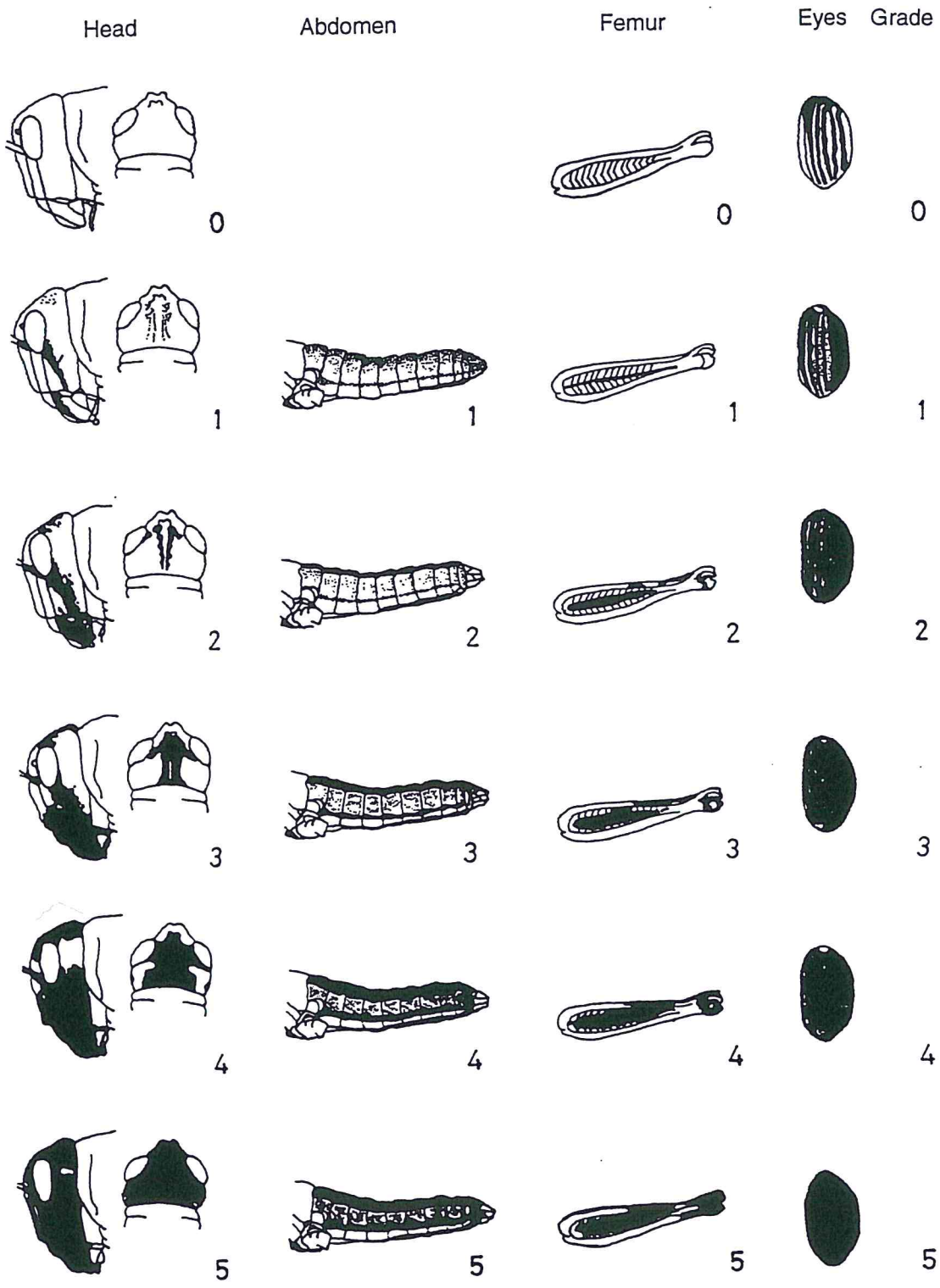

Figure 9. Black colour pattern used in grading nymphal colour changes in test *S. gregaria*

I

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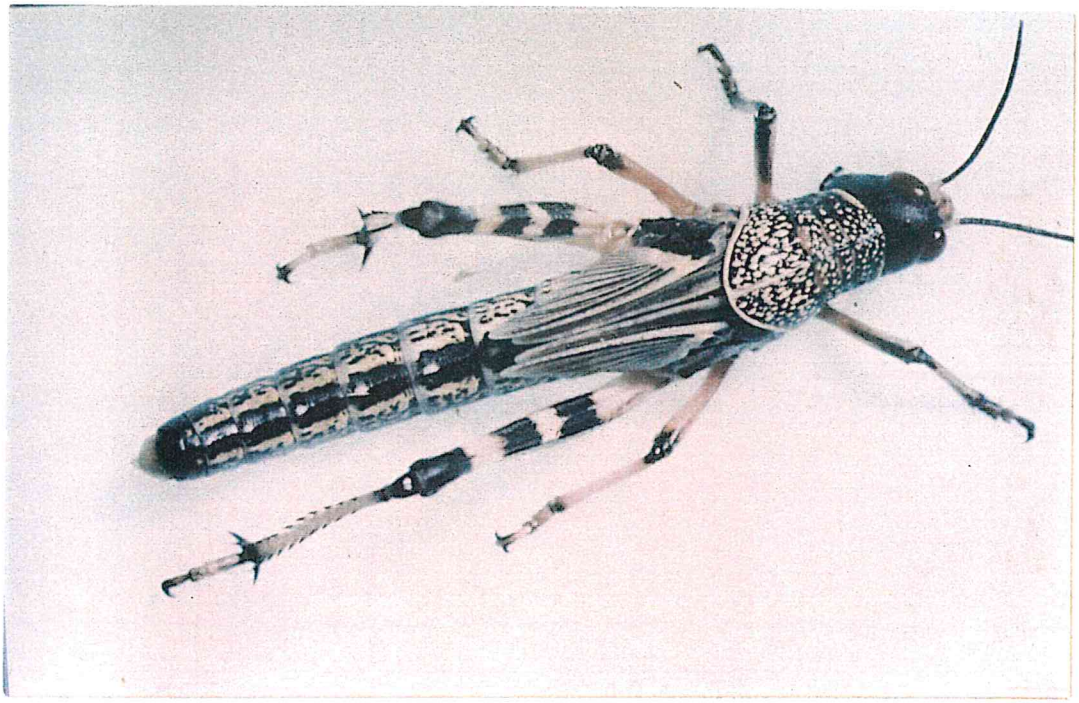

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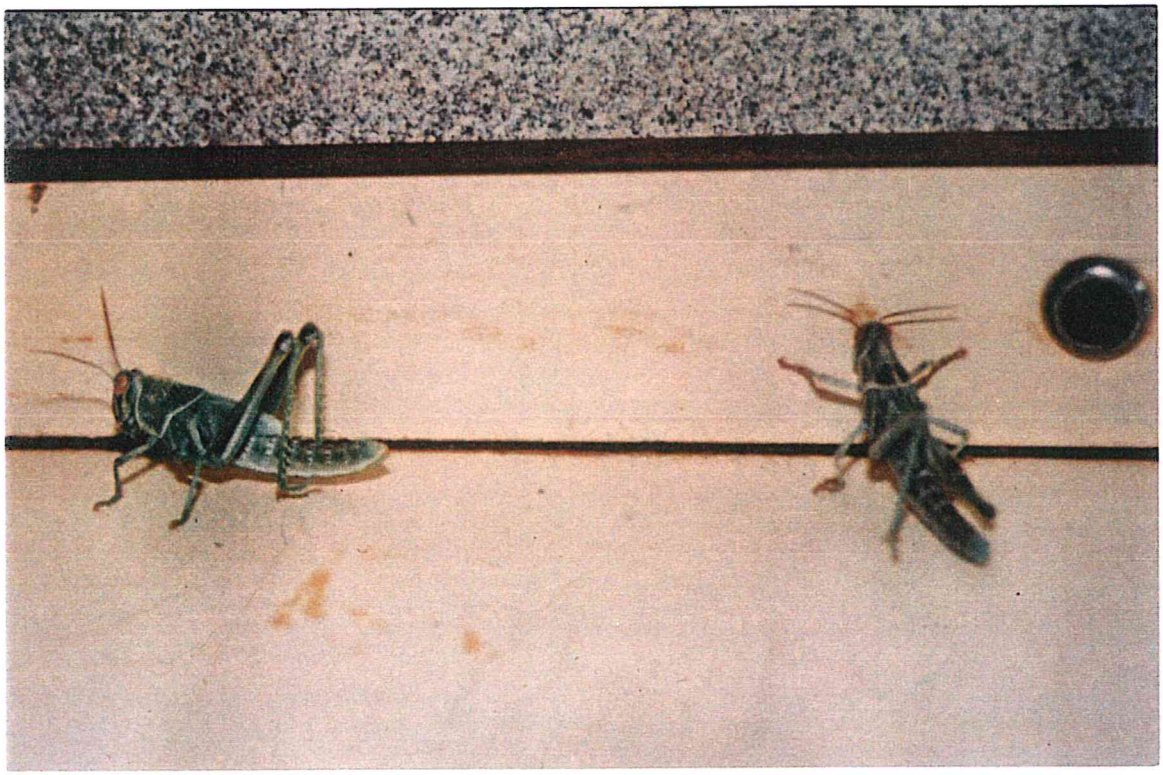


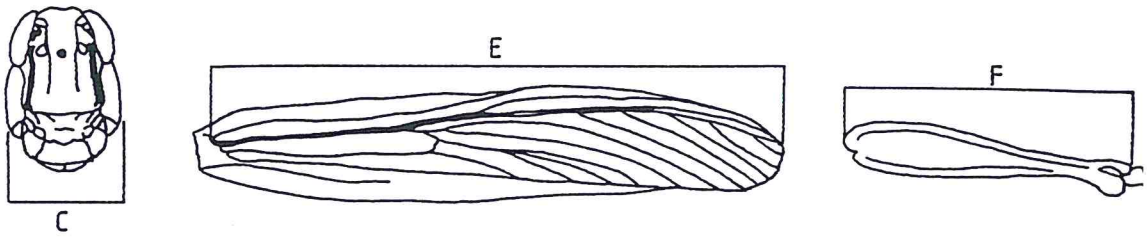
I. Gregarious fifth-instar and fourth-instar nymphs of *S. gregaria* from gregarious (I-1) and solitarious(I-2) control cages respectively.

the eye stripes of adult locusts. The duration of each instar was the time between consecutive moults whereas the nymphal developmental time was computed as the total of all instar durations. The total developmental time to end of immature stage was computed as the total nymphal developmental time added to the time the adult lived as immature.

Morphometric measurements

The length in millimeters (mm) of the elytron (E), the hind femur (F), and the width of the head capsule (C), were measured on mature adult males and females of both species using an electronic Sylvac (T) caliper with a range of 0-150 mm and an accuracy of ± 0.03 mm. Morphometric changes were determined by calculating E/F and F/C ratios (Fig. 10).

Average morphometric ratios were calculated and compared to those of control locusts to determine the phase status of all test locusts. To determine the individual phase status and the proportion of transformed individuals of the desert locust, a standard morphometric chart (Duranton and Lecoq, 1990) was used (Fig. 10).



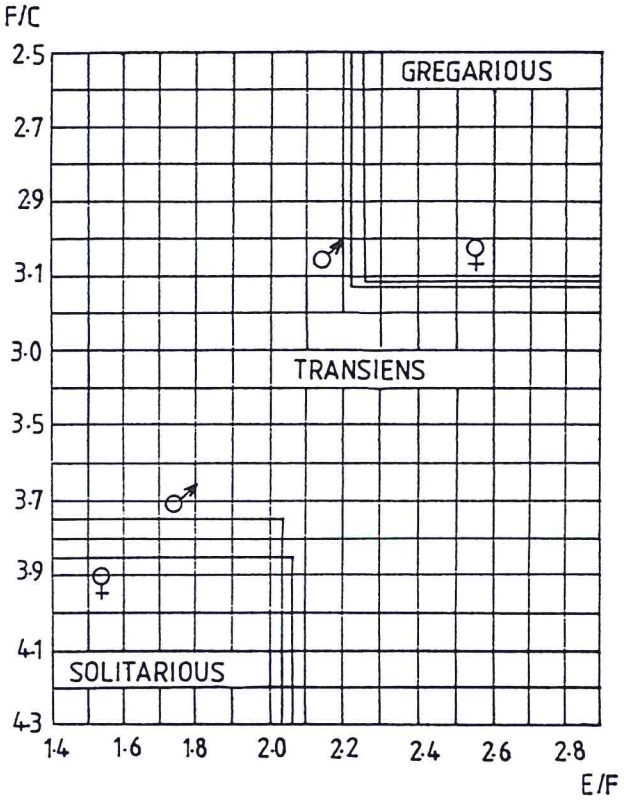


Figure 10. The standard morphometric chart for locust phase determination

Adult eye shading

The eye dark shading was recorded following Duranton and Lecoq (1990) grading scheme as follows: 0 : no shading of the eye, 1 : 1/3 of the eye shaded black, 2 : 1/2 shaded black, 3 : 1/2 shaded with additional black spots on the other 1/2, 4 : 2/3 shaded with additional spots, and 5 : eyes fully black coloured. Grade 0 corresponds to the solitarious phase, grade 3 to *transiens congregans*, and grade 5, fully gregarious desert locusts (Fig. 9).

Collection and analysis of haemolymph

Samples of locust haemolymph were collected from 33 day-old male and female adults of *S. gregaria*. This was done by puncturing the membrane at the precoxal cavity with a sterilized needle, and sucking the fluid up to 15 μl using microcapillary pipets (Sigma Chemical Co. St Louis Missouri). The sample fluid was diluted to 300 μl with phosphate buffer saline (PBS) pH 7.4 in Eppendorf tubes and kept at - 20°C until use (Mahamat et al. 1996).

The collected samples were analyzed for their uv-visible absorption spectra from 460 to 680 nm using a Beckman DU-50 UV Spectrophotometer with phosphate buffer saline as the reference (Mahamat et al., 1996). Absorbance ratios, R, were calculated as A1 / A2 where A1

was the absorbance reading at 460nm, and A2 the absorbance reading at 680nm.

Collection and analysis of volatiles

Air-borne volatiles were collected following the procedure described in Chapter III. Air from a compressed air cylinder was cleaned through a charcoal filter and passed over locusts placed in a 2 liter three-necked round bottomed flask. The volatiles released by the locusts in the air stream were adsorbed onto traps containing 60 mg of activated charcoal. After 16 hours (overnight), the traps were removed and eluted with 4 ml of dichloromethane (Aldrich Ltd., UK). The extract was then concentrated under a stream of nitrogen at 0°C to approximately 100 μl and stored at - 15°C prior to analysis. Collections were done on adult locusts at age 5-7, 10 - 12, 15 - 17, 20 - 22, 25 - 27, 30 - 32, and 35 - 37 days after fledging.

Four microliters were analyzed by capillary gas-chromatography (GC) and by GC-Mass spectrometry (MS). The columns used were methyl silicone SPB-1 (30m, 0.2 μm, and 0.2 mm ID), and Carbowax (50m, 0.2 μm, and 0.2 mm ID) (Chapter III, page 59). Nitrogen was used as the carrier gas at a flow rate of 0.35 ml/min.

5.2.2 Effects of gregarious *S. gregaria* on the gregarization of solitarious *L. migratoria*

Insects and experiments

Solitarious individuals of *L.m. migratorioides* were reared under conditions as described in Chapter III of this thesis. For this experiment only immature adults from the 3rd generation of isolated locusts were used. Locust isolated rearing was required, at least, for three generations, to make sure that they were solitary enough to be used in such experiments. It was impossible (because of low hatchability of eggs) to obtain a sufficient number of nymphs from mature adults of this generation to run this experiment at the nymphal stage.

Two-day-old immature adult solitarious locusts were placed in the cages as described above (Plate H) and reared with same age gregarious immature adults of *S. gregaria* to the end of the adult stage. Three treatments (TRT) were set up as follows:

TRT	GREGARIOUS STIMULUS	SOLITARIOUS TEST	RATIO	REPS
1.	Two-day-old immature adult *S. gregaria*	Two-day-old immature adult *L. migratoria*	80 : 1	9
2.	Two-day-old immature adult *L. migratoria*	Two-day-old immature adult *L. migratoria*	80 : 1	9
3.	Two-day-old immature *L. migratoria*	Two-day-old immature *L. migratoria*	0 : 1	9

Parameters monitored were integumental colour changes (on pronotum and frons) in immature and mature adults, and the amounts of phenylacetonitrile produced by maturing adult male locusts (Chapter III and Section 5.2.1).

5.2.3 Effects of volatiles of gregarious *L. migratoria* on the gregarization of solitarious *S. gregaria*

Insects and experiments

One two-day-old first instar solitarious desert locust was exposed to the volatile emissions from crowded (15) first-instar migratory locusts of similar age in a 15 x 15 x 30 cm bichamber cage (Plate A). Within each cage gregarious locusts placed in the top chamber, were visually separated from the solitary test locust by a black cloth placed at the bottom of the top chamber. Between cages, solitary locusts in the lower chamber were visually separated from each other by a black cloth taped over the window facing the neighbouring cage. Locusts were reared from nymphal to the adult stages. The following treatments (TRT) were set up:

TRT	GREGARIOUS STIMULUS	SOLITARIOUS TEST	RATIO	REPS
1.	First-instar *L. migratoria*	First-instar *S. gregaria*	15 : 1	6
2.	First-instar *S. gregaria*	First-instar *S. gregaria*	15 : 1	6
3.	First-instar *S. gregaria*	First-instar *S. gregaria*	0 : 1	6

Test locusts were monitored for nymphal integumental colour, number and duration of instars (nymphal developmental time), and adult integumental colour, morphometrics, duration of immature stage, and total developmental time (to the end of immature adult stage).

The colours of nymphs and adults were graded daily as described for nymphs (page 97) and adults (Chapter III, see page 62). The number and duration of nymphal instars were obtained from recording moulting periods, and counting adult eye stripes. Adult morphometrics were taken at age 12-15 days (Chapter III,) after fledging when locust integument was expected to have fully hardened.

5.3 Data analysis

Data were analyzed using SAS (1988). The data were transformed into log(x) or square roots, and means were separated, after analyses of variance, using LSD-test at $P \leq 0.05$.

5.4 Results

5.4.1 Effects of the presence of gregarious *L.m migratorioides*, and two grasshopper species on the gregarization of solitarious *S. gregaria*.

Experiment 1. Effects of the presence of nymphal *L. migratoria* on the gregarization of nymphal solitarious *S. gregaria*

Integumental colour

Integumental colour patterns in all nymphal desert locusts varied from green (grade 0 to 1) to yellow and black (grades 4 and 5) when reared mixed (singly) with gregarious migratory locusts. Nymphs changed to gregarious colour by the third instar, about 15 days after rearing of the two locust species together (Plate J; Table 6). About 13 (9 males and 4 females) out of the 19 desert locusts reared with each other reached grade 4 colour pattern and above by the fifth instar, representing a shift of 69% (75% in males, and 57% in females)(Table 6). These nymphs monitored to the pink fledglings attained stage III yellow colour at 29 ± 2 days (Plates C-1; D-1). The appearance of the yellow colour (Plate K) was, however, somewhat delayed by 7 days compared to gregarious controls.

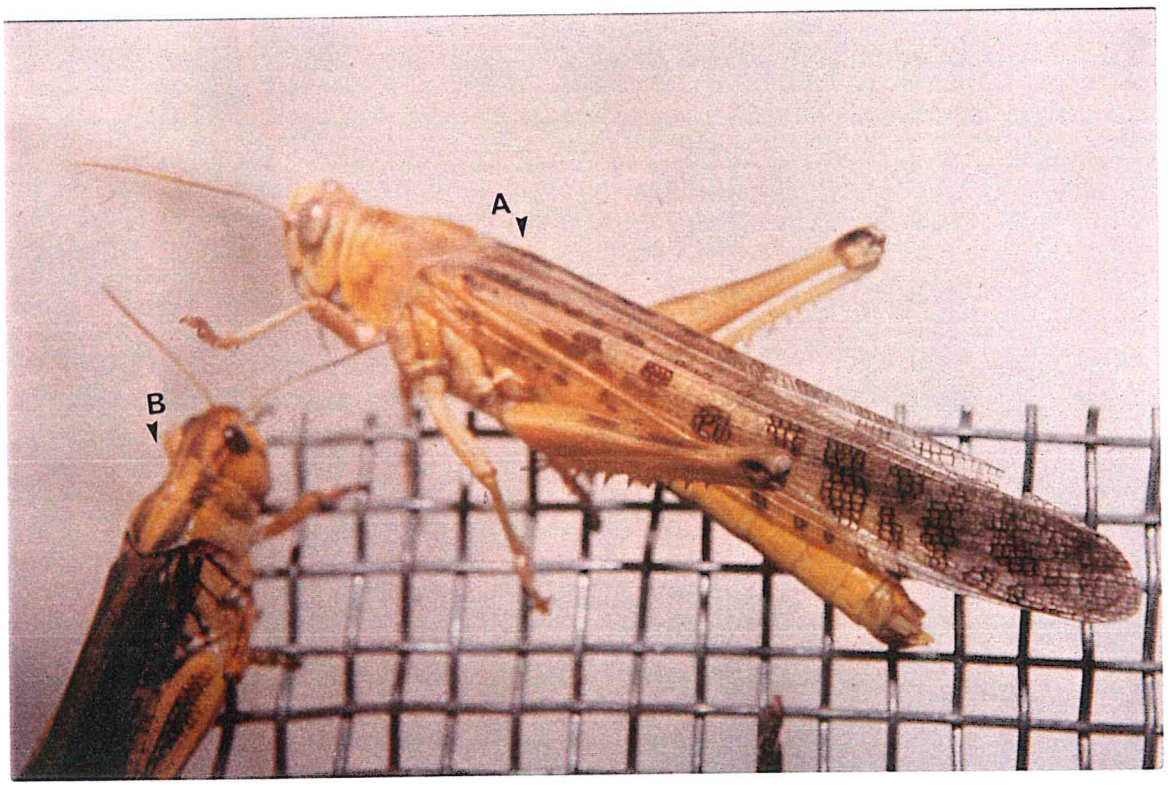
Number of nymphal instars, nymphal and total developmental times (to mature adult stage) times.

Solitarious *S. gregaria* reared with gregarious *L. migratoria* went through five or six nymphal instars. Only 50% (44% in males and 6% in females) of them went through five nymphal instars similar to the gregarious control while the remaining 50% (56% in males and 94% females went through six instars similar to the solitarious control. These results show that males were more responsive to the treatments and, therefore, transformed faster than females.

J

J. Solitarious *S. gregaria*(A) reared with gregarious *L. migratoria migratorioides*(B).

K



K. Adult male *S. gregaria*(A) reared with crowded *L. migratoria*(B) and showing yellow colour typical of mature gregarious locust

Table 6. Time (days) taken by test *S. gregaria* (SG) and *L. migratoria* (LM) to attain gregarious colour, and the corresponding percent shift when reared mixed with or exposed to volatiles

Source		Test locust		Rearing	Days(± SE) in Test locusts	Days (± SE) in control locusts	% shift in test locusts
Nymphs	LM	Nymph	SG	mixed	15.0 ± 2 a	4.0 ± 2 a	69 b
Nymphs	PV	Nymph	SG	mixed	17.0 ± 2 a	4.0 ± 2 b	63 b
Nymphs	EP	Nymph	SG	mixed	-	4.0 ± 2	0 c
Nymphs	LM	Adult	SG	mixed	20.0 ± 0.6 b	22.4 ± 0.6 a	90 a
adult	LM	Adult	SG	mixed	15.3 ± 0.3 b	22.4 ± 0.6 a	89 a
adult	SG	Adult	LM	mixed	10.3 ± 0.3 b	11.8 ± 0.4 a	100 a
Nymphs	LM	Nymph	SG	exposure	25.0 ± 3.0 a	22.4 ± 0.6 b	100 a

Treatment and control means in each experiment followed by the same letter are not significantly different (at 5 % level; Student T-test). Percent(%) shifts(to gregarious colour) in last column were compared between experiments.
PV refers to the grasshopper *P. viridipes* and EP to *E. plorans.*

Fifth and sixth instars were the longest while those of the other instars were quite variable compared to gregarious and solitarious controls. Solitarious *S. gregaria* reared with gregarious *L. migratoria* took 13 to 19 days longer than the gregarious and solitarious controls, respectively, to complete their nymphal stage. There was, however, significant similarity ($P<0.05$) in development between nymphs from the test and those from the control colonies, especially at second, and from the fourth, fifth, and sixth-instars (Fig. 11). There significant synchrony in developmental time between the test desert locusts and the source migratory locusts (Fig. 12)

The total nymphal developmental time of the test locust was 46 days and immature adult took 23 days to reach maturation making it a total developmental time to mature adult of about 79 days.

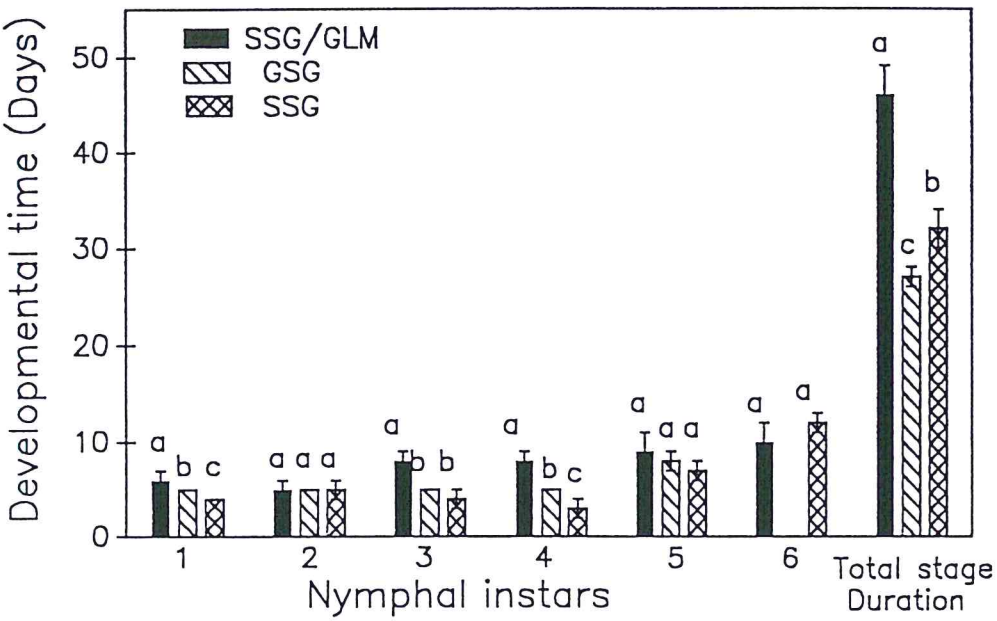


Figure 11. Developmental times of solitarious test *S. gregaria*(SSG) reared with gregarious *L. migratoria*(GLM), **[(SSG/GLM)]** compared to gregarious **[(GSG)]** and solitarious **[(SSG)]** controls.

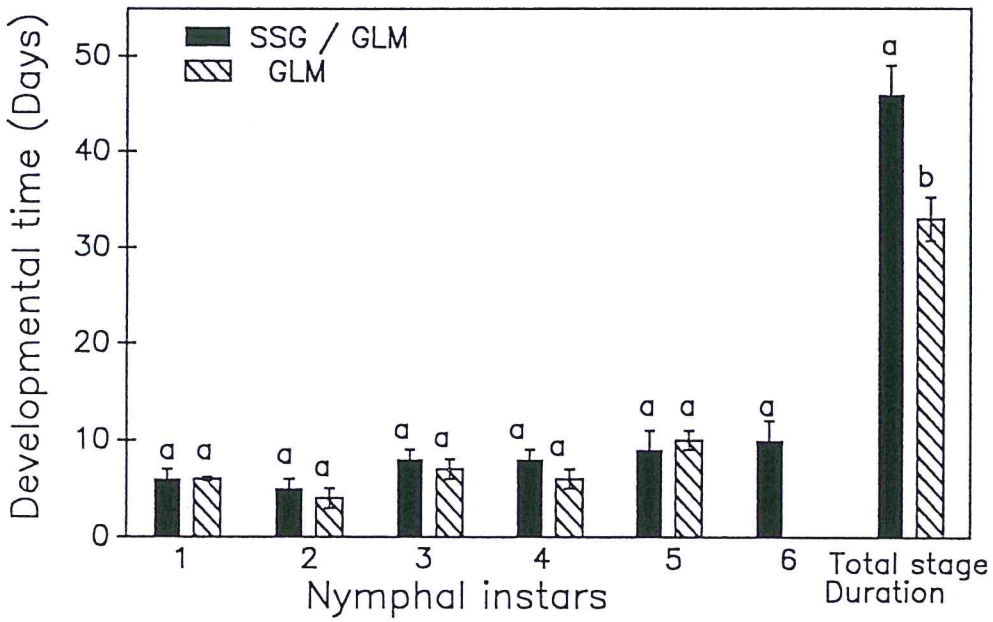


Figure 12. Nymphal instar and stage durations of solitarious test *S. gregaria*(SSG) reared with gregarious *L. migratoria*(GLM), **[(SSG/GLM)]** and that of those which it was reared with**[(GLM)]**.

Adult morphometrics

Average E/F ratios showed that test *S. gregaria* males were more like solitarious while females were more like the gregarious controls. Average F/C ratios showed that test female locusts were all transients (Fig. 13). However, fewer males than females transformed to the gregarious phase.

Comparison with the standard morphometric chart showed that three out of the eleven males *S. gregaria* reared with *L. migratoria* changed to transiens while eight remained solitarious, representing a percent shift of 27% to transient phase. In females, only three out of eight changed into transients (37% shift) by the end of the first generation.

Eye shading

Nine (8 males and 1 female) out of sixteen solitarious adult desert locusts which were reared singly with gregarious migratory locusts attained grade 3 eye shading representing a total shift to the gregarious phase of 57%

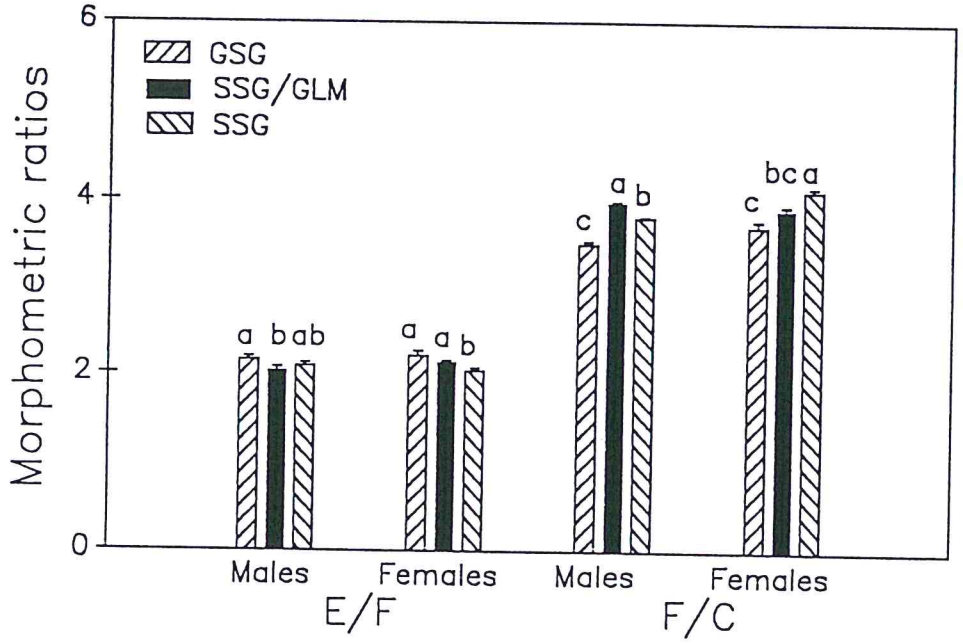


Figure 13. Adult E/F and F/C ratios of solitarious *S. gregaria*(SSG) reared with gregarious *L. migratoria*(GLM) from nymphal stage **[(SSG/GLM)]** compared to gregarious**[(GSG)]** and solitarious controls**[(SSG)]**.

(67% in males, and 15% in females). Males were more responsive to treatment effects than females, and therefore, changed faster.

Haemolymph pigments

Haemolymph absorbance ratios (pigment composition) of all test desert locusts *S. gregaria* was intermediate between isolated individuals and crowded controls, especially in females (Fig. 14).

Volatile emissions

Volatile emissions from test *S. gregaria* mature adult males contained phenylacetonitrile similar to control gregarious mature adult locusts (Figs. 15;16). Its production followed the same pattern in both control and test insects, but reaching an optimum between 15 - 20 days after fledging in controls, and 25 - 30 days in test insects (Fig. 17). Phenylacetonitrile was also detectable in the volatile emissions of mature adult males of gregarious *L. migratoria* (Fig. 18) which test locusts were reared with.

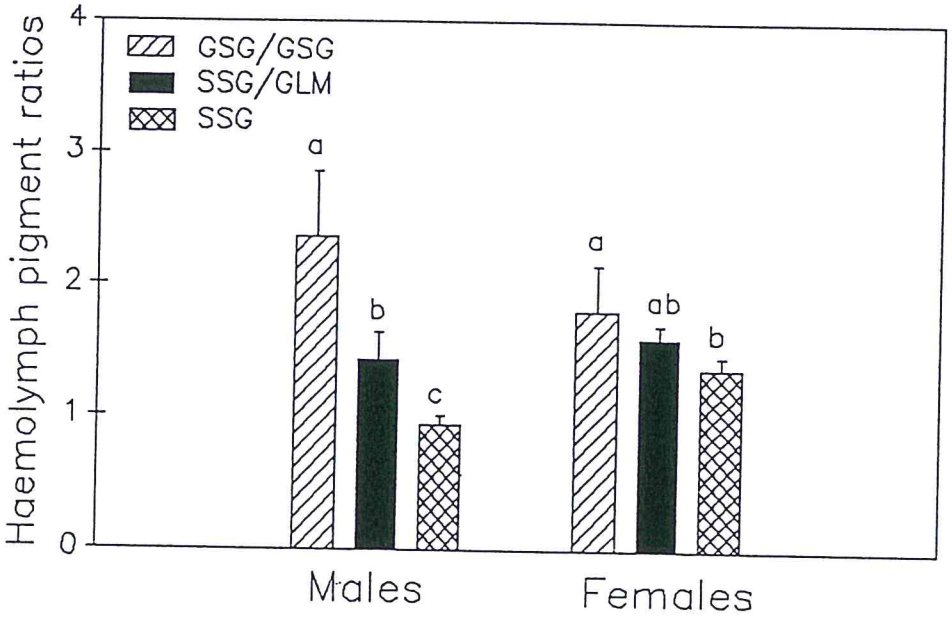


Figure 14. Haemolymph pigment ratios(at 460 over that at 680 nm wavelength) of test *S. gregaria*[(SSG/GLM)] compared to gregarious[(GSG)] and solitary[(SSG)] controls.

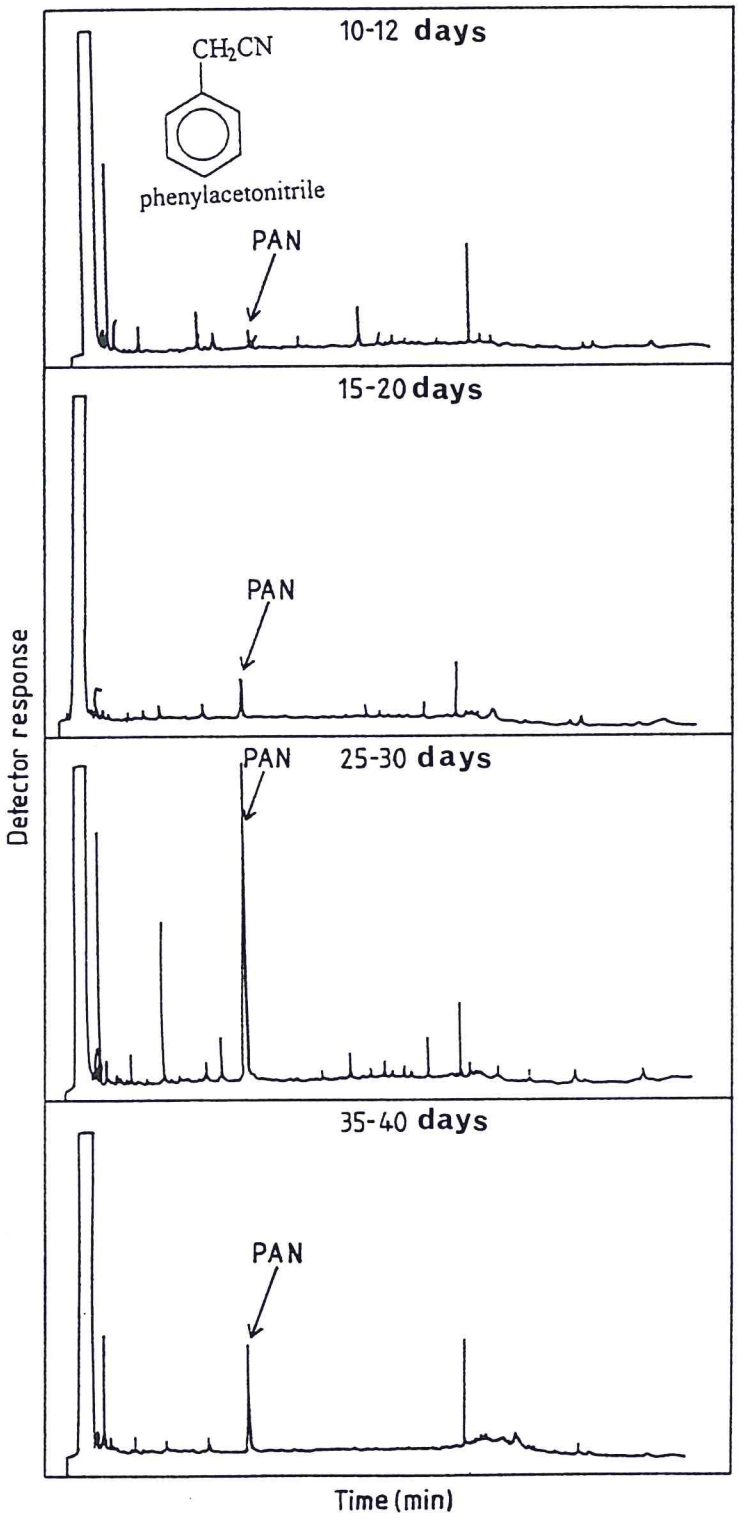


Figure 15. Gas chromatograms showing the production of phenylacetonitrile (PAN) in adult males of *S. gregaria* reared with gregarious *L. migratoria* at 10-12, 15-20, 25-30, and 35-40 days after fledging (30m, 0.2mm ID methyl silicone column) .

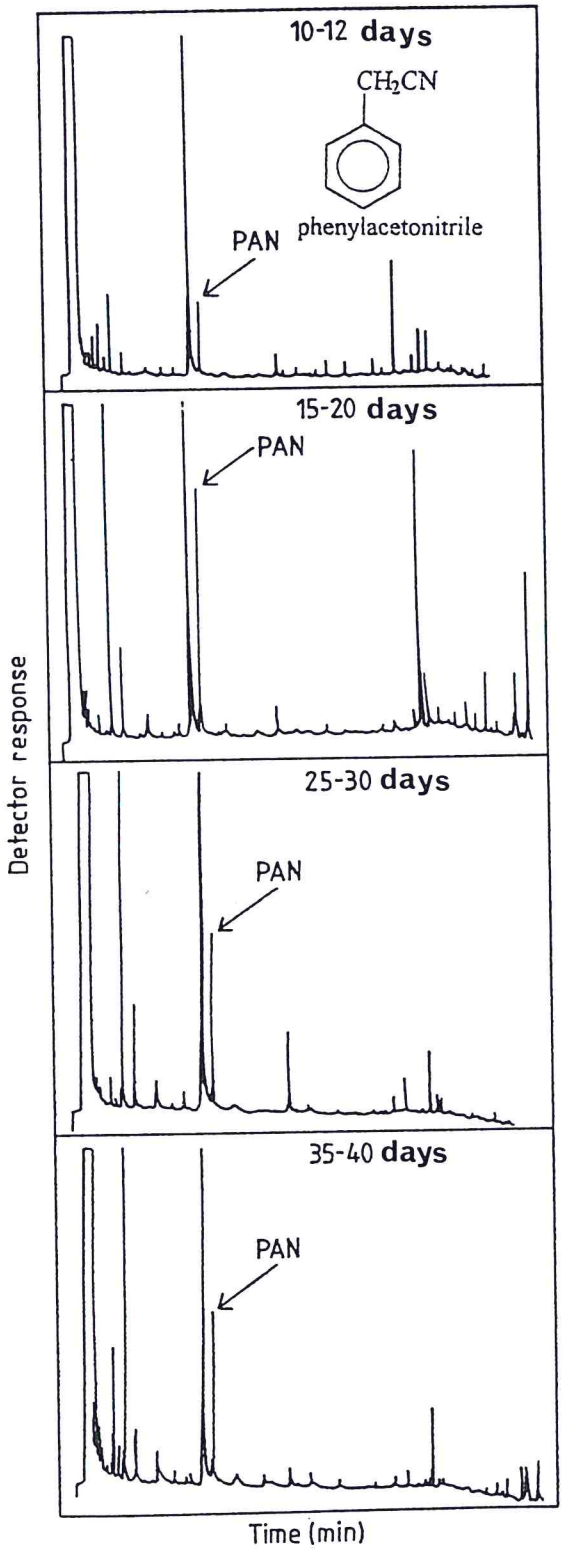


Figure 16. Gas chromatograms showing peaks of phenylacetonitrile (PAN) produced by gregarious locusts from control cages at 10-12, 15-20, 25-30, and 35-40 days after fledging (30m, 0.2mm ID methyl silicone column)

This compound was produced in relatively smaller proportions in adults of this species compared to the emissions from test and control mature adult males of *S. gregaria*.

Experiment 2. **Effects of the presence of immature adults of *L. migratoria* on the gregarization of immature solitarious adults of *S. gregaria***

Integumental colour

Both male and female solitarious immature adults of *S. gregaria* reared with crowded immature adults of *L. migratoria* attained stage III yellow colour 15 days of mixed rearing representing a shift to gregarious phase of 89% (all males and 67% of females) of the test insects (Plate L). Immature adult males were more responsive to treatment effects than females (Table 6).

Volatile emissions

Solitarious immature adult males of *S. gregaria* reared with gregarious immature adults of *L. migratoria* produced phenylacetonitrile after 10 days of rearing with maximum

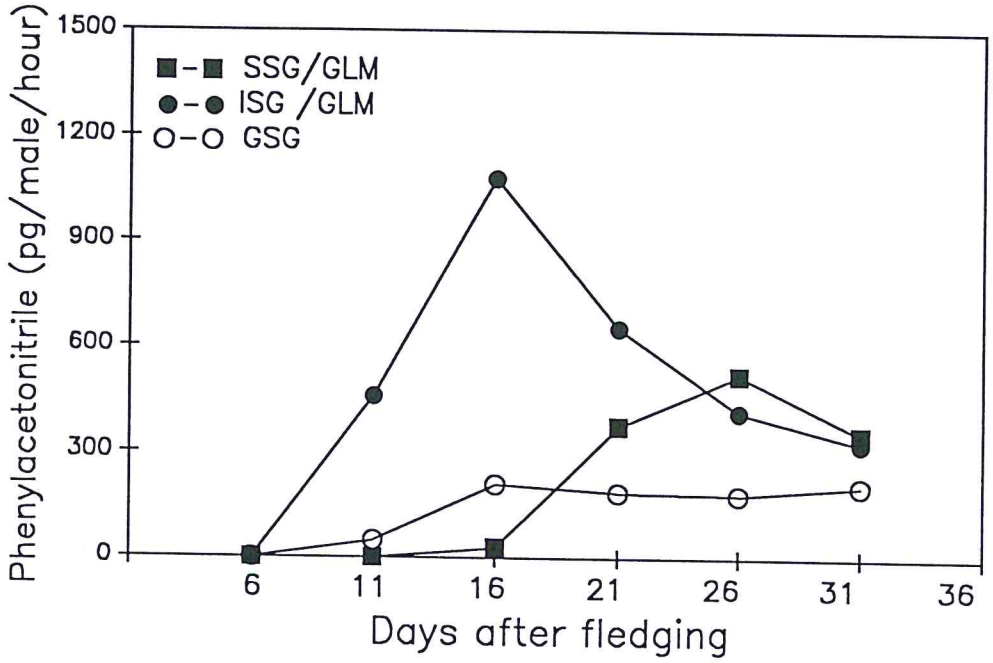


Figure 17. Phenylacetonitrile titres (pg) produced by *test S. gregaria*(SSG) when reared with gregarious *L. migratoria*(GLM)at nymphal stage**[(SSG/GLM)]** and immature adult **[(ISG/GLM)]** as compared to titres of conspecifics from gregarious control**[(GSG)]**.

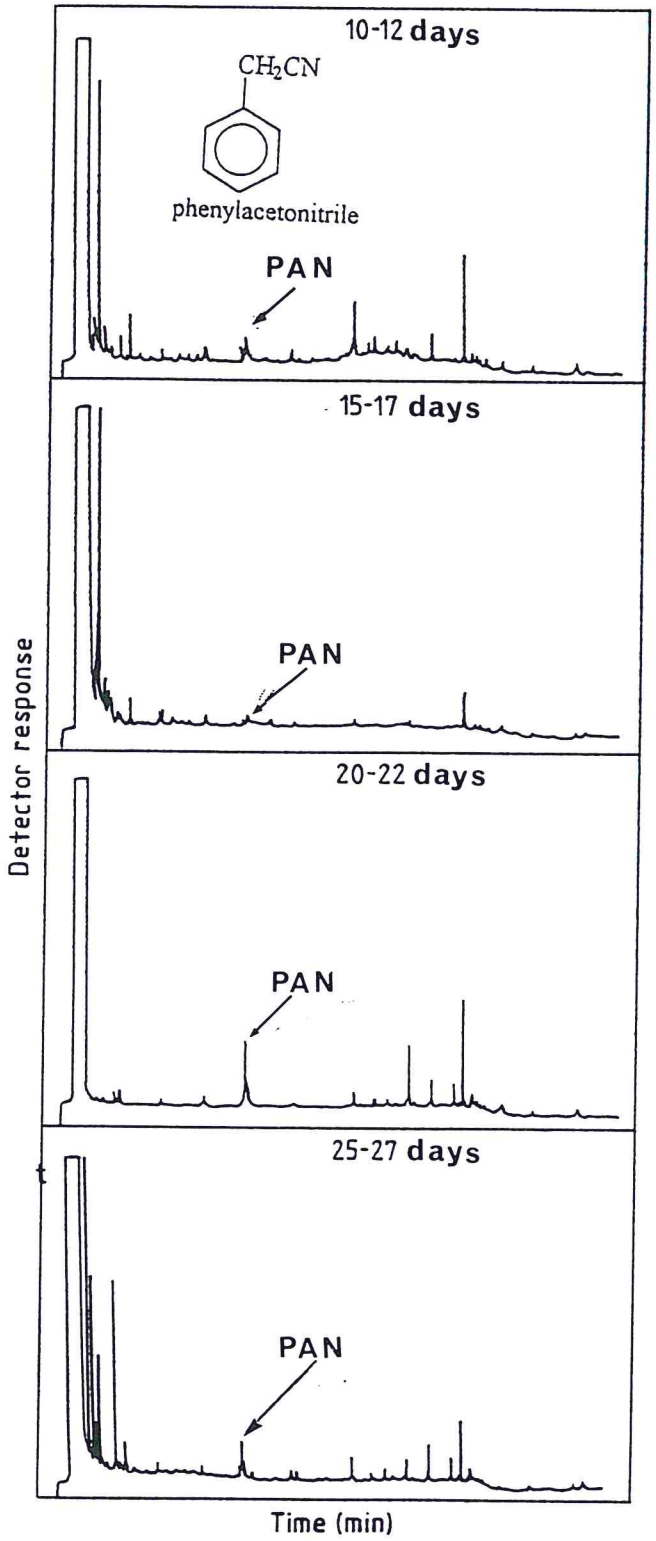


Figure 18. Gas chromatograms showing the production of phenylacetonitrile (PAN) in gregarious mature adults of *L. migratoria* at 10-12, 15-17, 20-22, and 25-27 days after fledging (30m, 0.2mm ID SPB-1 methyl silicone column)

production occurring between 15 to 17 days, similar to gregarious control counterparts (Fig. 17).

Eye shading

Seven (4 males and 3 females) out of the nine test desert locusts attained grade 3 eye shading stage and above by the end of the experiment, representing a shift of 78% (80% males and 75% females).

Experiment 3. Effects of the presence of fifth-instar nymphs of *L. migratoria* on immature adults of *S. gregaria*

Integumental colour

Solitarious male and female immature desert locusts reared with fifth-instar nymphs of the migratory locusts attained stage III yellow colour 20 ± 0.6 days after fledging representing a shift of 90% (80% in males and 100 % in females)(Table 6).

Volatile emissions

Solitarious immature desert locusts reared with fifth-instar nymphs of the migratory locusts produced trace of phenylacetonitrile about 15 days after fledging (Fig. 19).

Eye shading

Solitarious immature desert locusts which were reared with fifth-instar nymphal migratory locusts attained grade 3 eye shading stage typical of transient or gregarious phase. All males and females were in transient phase representing a 100% shift.

Experiment 4. Effects of the presence of nymphs of *P. viridipes* on the gregarization of nymphal *S. gregaria*

Integumental colour

About 63 % (75% in males and 75% in females) of nymphs reared with *P. viridipes* attained gregarious colour characteristics by the 4th-instar, 17 days after rearing

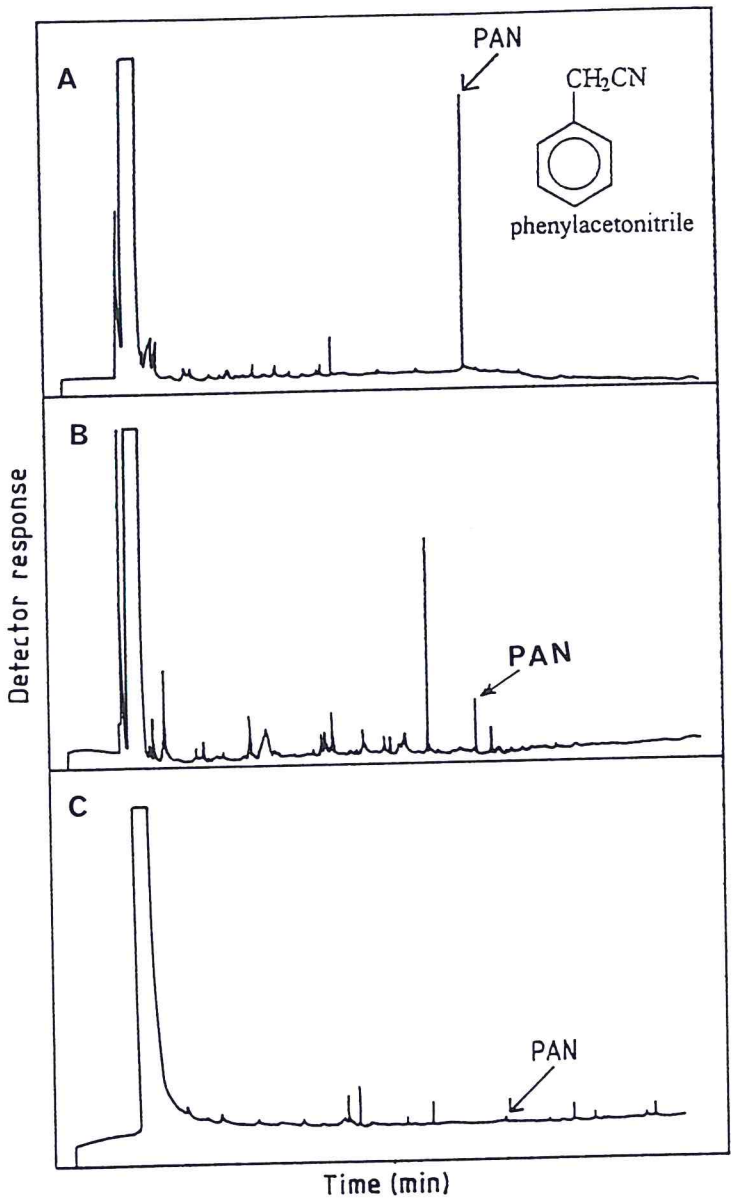


Figure 19. Gas chromatograms showing A) peaks of phenylacetonitrile (PAN) produced by *S. gregaria* reared from immature solitary adult stage with gregarious adults of *L. migratoria* ; B) produced by *L. migratoria* reared from immature solitary adult stage with gregarious adults of *S. gregaria*; and C)produced by adults of *S. gregaria* reared from immature solitary adult stage with fifth-instar nymphs of *L. migratoria*(50m, 0.2mm ID Carbowax column).

(Plate L;Table 6). Like in previous mixed rearing from nymphal stage (Experiment 1), gregarious colour in nymphs was observed to intensify after each moult, during the shifting process.

Number of nymphal instars, nymphal and total developmental time (to immature adult stage)

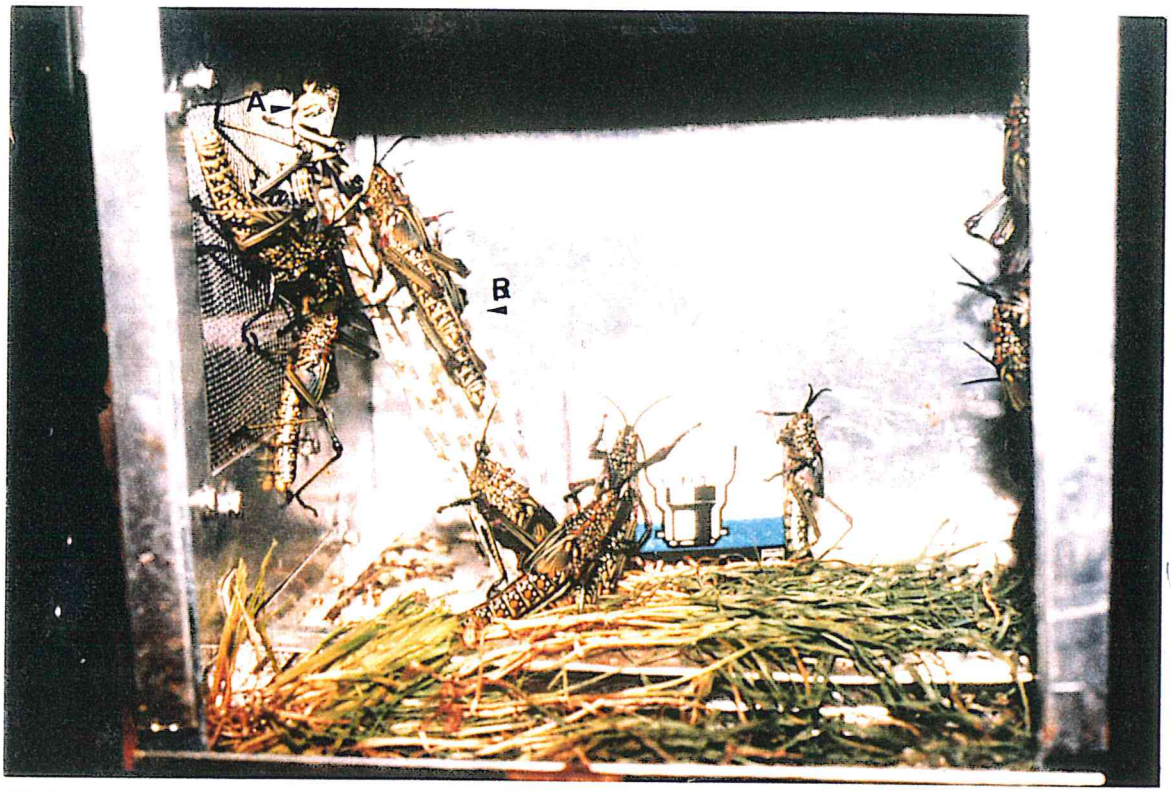
Solitarious *S. gregaria* reared with *P. viridipes* went through five or six nymphal instars. Fifty percent of them went through five nymphal instars (75% in males only) like in gregarious controls, while the other 50% went through six nymphal instars (females only) like solitarious controls (Table 7). Developmental times for first and sixth instars were the longest followed by the third, fourth and fifth instars. The second instar developed fastest (4 days). Compared to the control gregarious and solitarious locusts, the durations of the instars of the test *S. gregaria* were variable (Table 7).

Test nymphal desert locusts took a total of 45 ± 3 days to complete development. This represents a delay of about 15 days compared to the nymphal development in gregarious and solitarious controls (Table 7).

Fifty percent of the fledglings attained stage III yellow colour typical of mature gregarious locusts after 35

L

1



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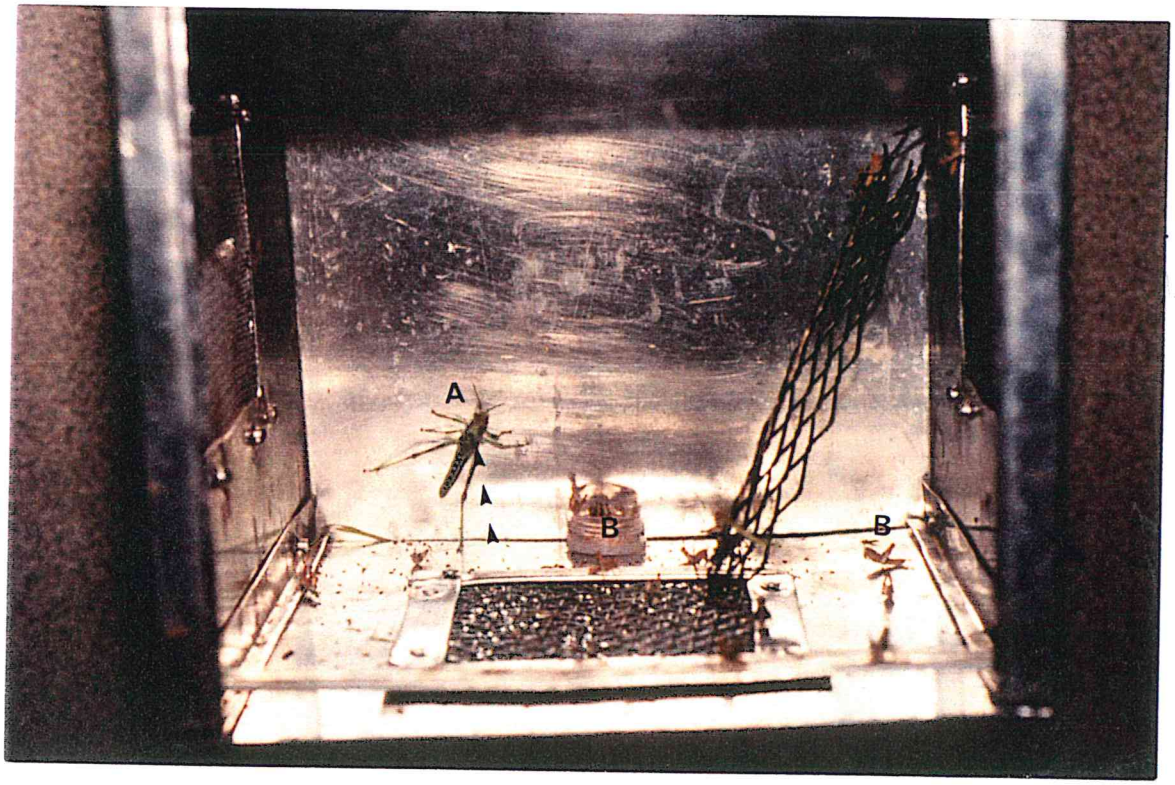

L. Solitarious *S. gregaria*(A) reared with *P. viridipes*(B)(L-1), and *E. plorans*(B) (L-2) from nymphal stage.

Table 7. Developmental time (days) of isolated S. gregaria(SSG) reared with P. viridipes (PV),[(PV/SSG)] and E. plorans(EP), [(EP/SSG)] as compared to those from gregarious[(GSG/GSG)] and solitarious (1 SSG/cage) controls.

Source/Test	1st-instar	2nd-instar	3rd-instar	4th-instar	5th-instar	6th-instar	Total stage	Young adult stage	Total time to mature
PV/SSG	15±1a	4±1bc	7±1b	9±1a	9±1b	11±2a	45±3a	54±10a	99±10a
EP/SSG	5±1cd	6±1a	9±0ab	6±2b	12±1a	9±1a	47±3a	8±1c	
GSG/SSG	5±1cd	5±1ab	5±1c	5±1b	8±1b	-	27±2c	22±1b	55±3c
1SSG/cage	4±1d	5±1ab	4±1c	3±1c	7±1b	12±1a	32±1b	-	74±2b
									-

In columns, means followed by the same letter are not significantly different(at 5% level; LSD-test).

days. The remaining 50% stayed pink by the conclusion of the experiment (55-60 days after fledging).

Morphometrics

E/F ratios of males of test *S. gregaria* were closer to solitarious controls while females were intermediate between solitarious and gregarious controls. F/C ratios of males were more were more like solitarious and females were transients (Fig. 20). Comparison with the standard morphometric chart (Duranton and Lecoq, 1990) showed that test locusts were more like transiens, a shift of about 67% (75% in males and 50% in females).

Volatile emissions

Mature adult males of *S. gregaria* which fledged from nymphs reared with *P. viridipes*, produced trace amounts of phenylacetonitrile in their volatile emissions 15 and 25 days after fledging.

Analysis of the volatiles from *P. viridipes* showed the presence of benzaldehyde, phenol, and guaiacol three of the aggregation pheromone components of mature desert locust.

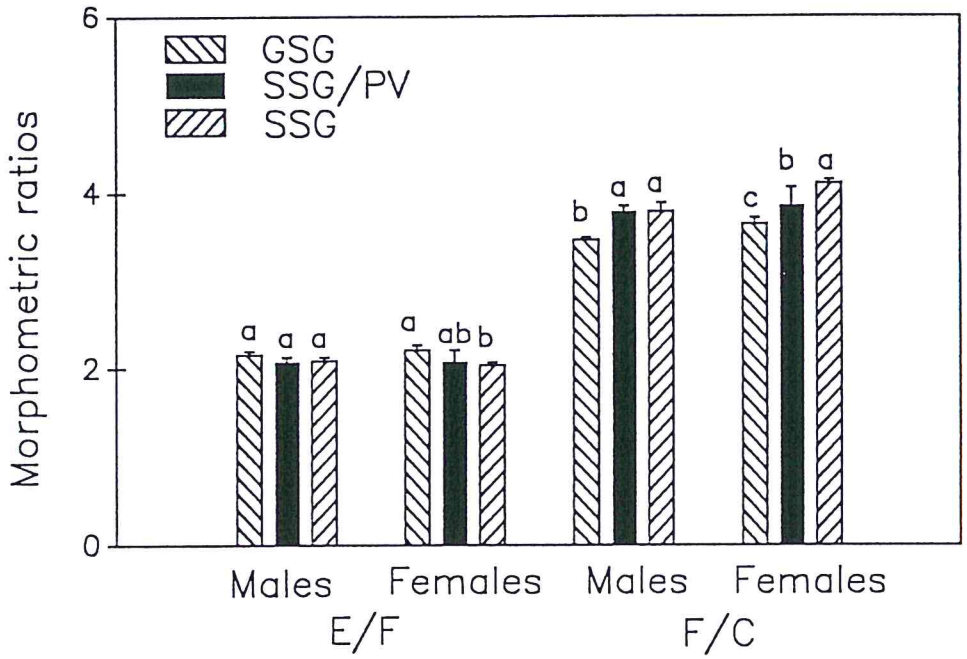

Figure 20. Adult E/F and F/C ratios of solitarious *S. gregaria* (SSG) reared with *P. viridipes* (PV) at nymphal stage **[(SSG/PV)]** compared to gregarious **[(GSG)]** and solitarious **[(SSG)]** controls.

These compounds are also present in the volatile emissions of the migratory locust (Appendix VII) .

Eye shading

Five out of the six monitored desert locusts reared with *P. viridipes* showed significant dark shading of the eye of grade 3 and above, similar to those reared with *L. migratoria* and the control gregarious colony. This represents a shift of 84% (all males and 75% of females).

Experiment 5. Effects of the presence of nymphs of *E. plorans* on the gregarization of nymphal solitarious *S. gregaria*

Nymphs of solitarious *S. gregaria* reared with *E. plorans*, maintained the colour characteristics of the solitarious controls (nymphs green and adults grey colour). Six nymphal instars were recorded and the total nymphal developmental time was up to 47 days (Table 7). Adults (which live for 8 days as immature) had eye shading and morphometrics typical of solitarious locusts (Fig. 21), and did not produce phenylacetonitrile.

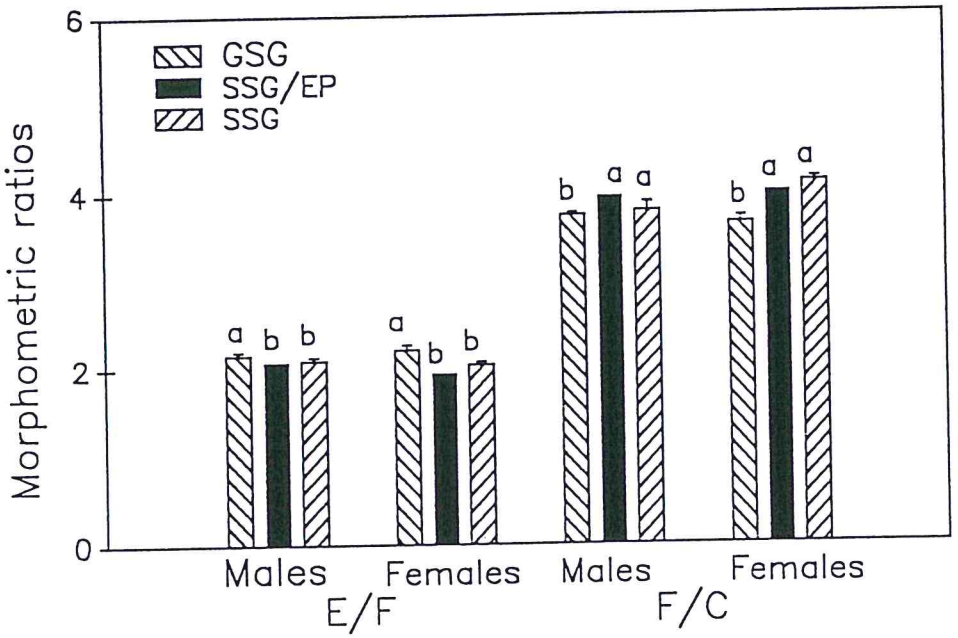

Figure 21. Adult E/F and F/C ratios of solitarious test *S. gregaria* (SSG) reared with *E. plorans*(EP) at nymphal stage**[(SSG/EP)]** compared to gregarious**[(GSG)]** and solitarious**[(SSG)]** controls.

5.4.2 Effects of gregarious *S. gregaria* on the gregarization of solitarious *L. migratoria*

Immature adults of *L. migratoria* which were reared with gregarious immature adults of *S. gregaria* progressively developed brown to black colour on pronotum, thorax, and legs. At the same time, the green colour on these body parts faded. Mature adults showed yellow colour on frons, typical of gregarious locusts (Table 6). Changes in colour to gregarious form occurred in about 10 days after fledging, which was about 5 days faster than observed for solitarious *S. gregaria* reared with gregarious *L. migratoria* at the immature adult stage. All test migratory locusts had changed to gregarious phase at the end of the experiment.

Mature adult males of test *L. migratoria* produced significantly higher amounts of phenylacetonitrile than gregarious conspecific locusts in the control (Fig. 22). The maximum amounts of this compound in test *L. migratoria* occurred 15 days after fledging while in the control, it occurred after 20 days.

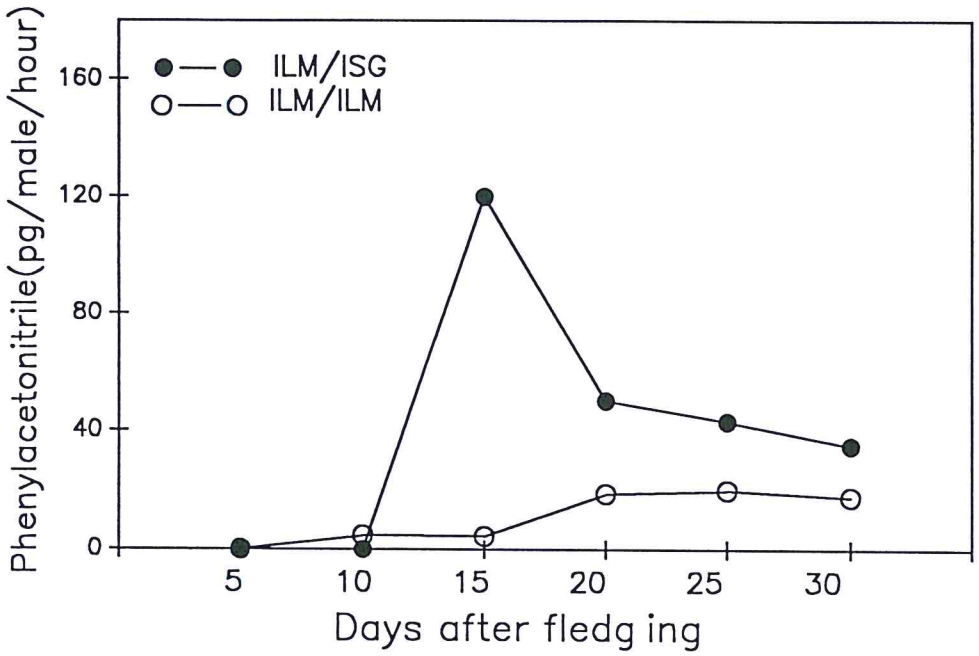

Figure 22. Production(in pg) of phenylacetonitrile by solitary immature *L. migratoria*(ILM) when reared with gregarious immature adults of *S. gregaria*(ISG), **[(ILM/ISG)]** compared to those reared with gregarious conspecific controls **[(ILM/ILM)]**.

5.4.3 Effects of volatiles of gregarious *L. migratoria* on the gregarization of solitarious *S. gregaria*

Integumental colour

Nymphs exposed to volatiles of *L. migratoria* attained the gregarious colour grade 3 in 25 ± 3 days (Table 6). All locusts tested shifted to gregarious colour and nymphs fledged into pale pink immature adults and attained stage III yellow colouration at maturity.

Number of instars, nymphal and total developmental times (to mature adult stage).

Eighty percent of solitarious test locusts went through 6 nymphal instars typical of the solitary phase, and only 25% (only males) went through 5 nymphal instars, typical of the gregarious phase. This represents a lower (by 25 %) percentage shift in comparison to that observed from mixed rearing with either *L. migratoria* (Experiment 1) or *P. viridipes* (Experiment 4). The instar duration pattern was irregular. The first and third instars were the longest (10 days) and the second the shortest (6 days) (Fig. 23). The total nymphal developmental time was significantly longer than that of those of gregarious and solitarious controls. There was no clearcut synchrony in nymphal development between nymphs of *L. migratoria* in

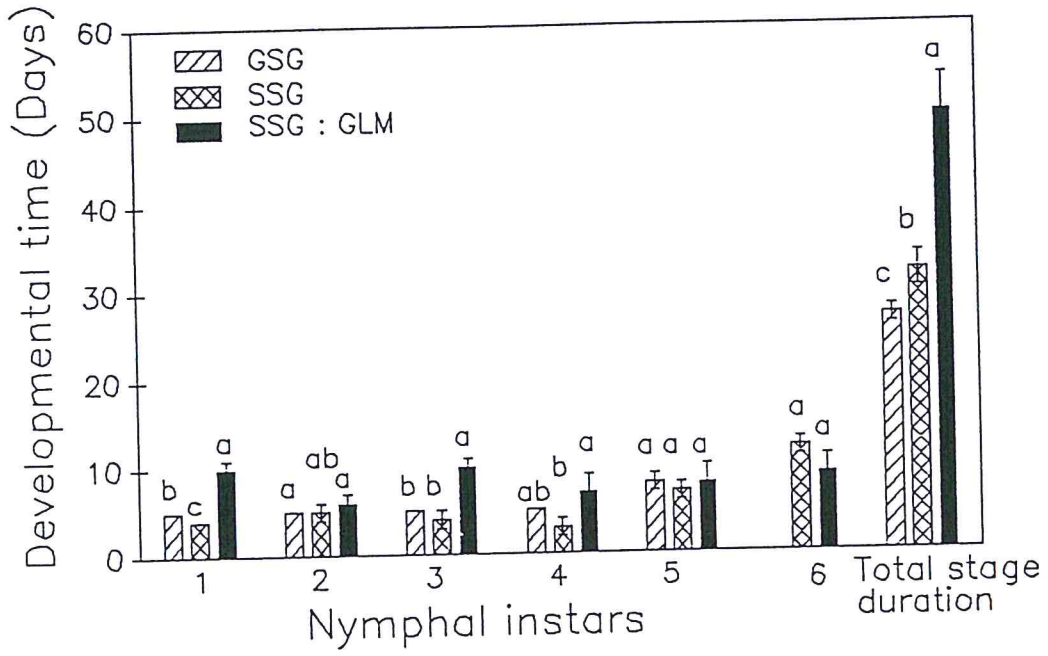

Figure 23. Nymphal instar and stage duration of test solitarious *S. gregaria*[**(SSG)**] exposed to volatiles of gregarious source *L. migratoria*[**(GLM)**] from nymphal stage stage[**(SSG:GLM)**].

the source and the recipient *S. gregaria* (Fig. 24).

Nymphal developmental time was 50 ± 4 days, immature adults attained stage III colour in 29 ± 4 days, leading to a total developmental time of 79 ± 6 days, similar to that of those reared with gregarious *L. migratoria* ($P \leq 0.05$) (Fig. 25).

Morphometric ratios

E/F ratios in males were intermediate between gregarious and solitarious controls whereas females were more like solitarious controls. The same pattern was observed with F/C ratios (Fig. 26). The standard morphometric chart suggests that about 50% (one half of males and one half of females) of the test *S. gregaria* were *transiens*, similar to those reared with *L. m. migratorioides* (Experiment 1), and *P. viridipes* (Experiment 4), but contrasted those reared with *E. plorans* (Experiment 5). Later in their development, five out of the six locusts studied had deformed (twisted) wings.

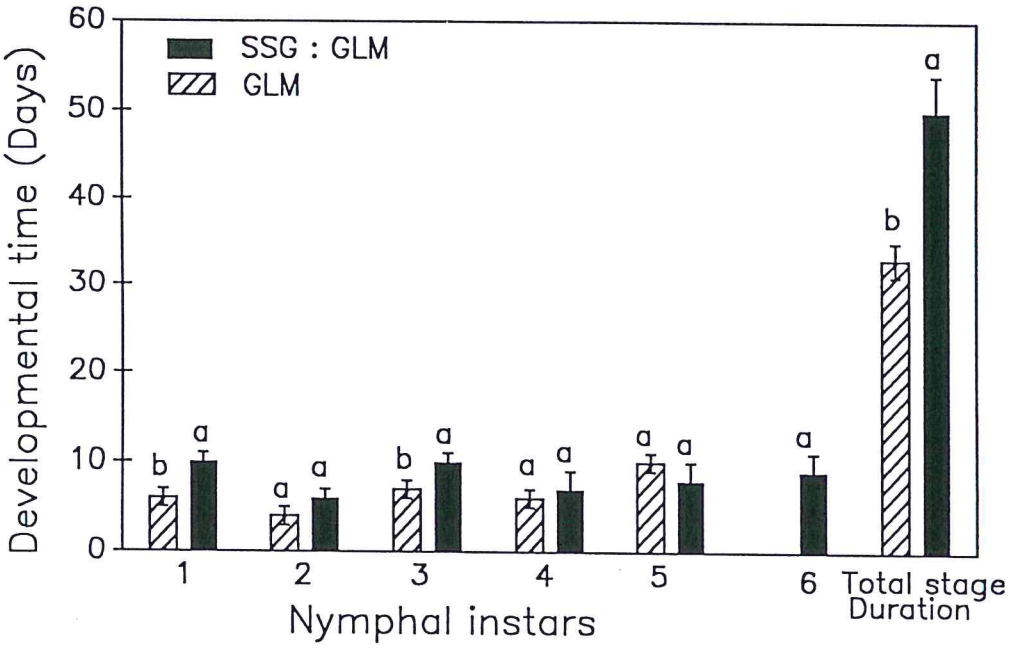

Figure 24. Nymphal instar and stage durations of solitarious test *S. gregaria*(SSG) exposed to volatiles of gregarious *L. migratoria* (GLM), **[(SSG:GLM)]** from nymphal stage compared to source gregarious *L. migratoria* **[(GLM)]**.

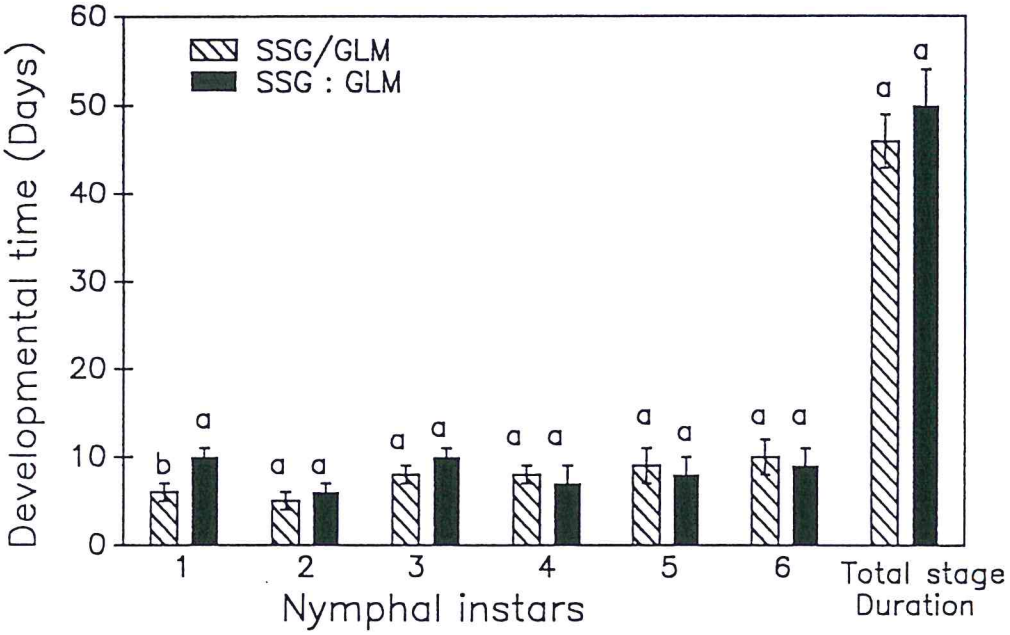

Figure 25. Nymphal instar and stage duration of test solitary *S. gregaria*(SSG) reared with gregarious *L. migratoria* (GLM), **[(SSG/GLM)]** or exposed to its volatiles **[(SSG:GLM)]**.

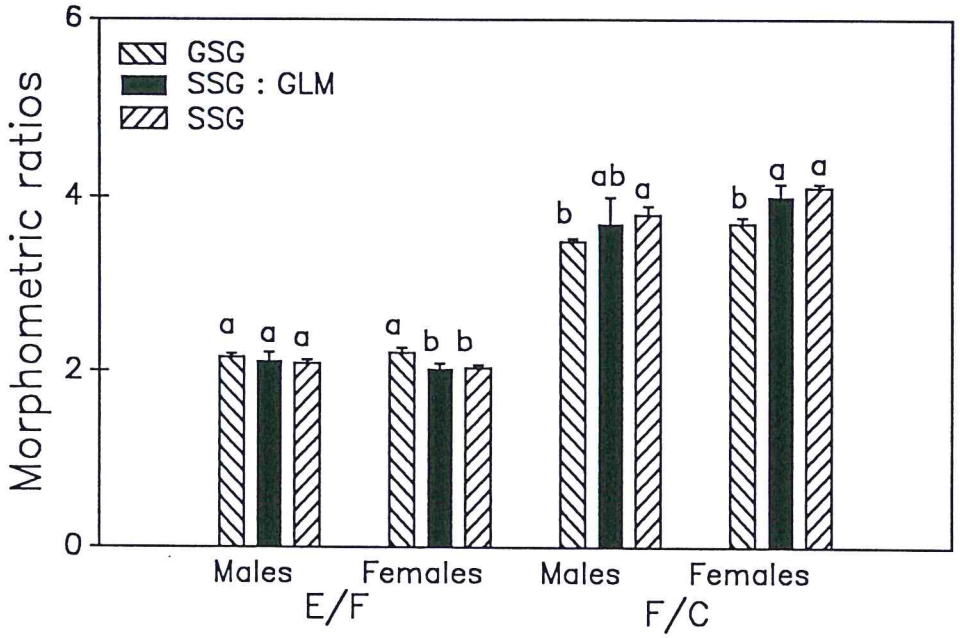

Figure 26. E/F and F/C ratios of solitary *S. gregaria*(SSG) exposed to volatiles of gregarious *L. migratoria*(GLM) from nymphal stage**[(SSG:GLM)]**.

Volatile emissions

Of the 10 starting replicates, three did not reach the fifth-instar stage due in part by ants preying on moulting nymphs, one died at fifth-instar nymphal stage (not preyed upon), and only one adult male survived up to 20 days after fledging, therefore, volatile collections were discontinued.

In summary, *S. gregaria* reared with *L. migratoria* and *P. viridipes* shifted towards gregarious characters. All locusts reared with other species than *S. gregaria* had an irregular nymphal development, leading to longer nymphal developmental time, with fledglings taking relatively longer period to attain stage III colour, compared to those from the control gregarious and solitarious cages (Fig. 27). Fledglings from nymphs reared with *E. plorans* did not reach stage III yellow colour since they died eight days after fledging. Fledglings from locusts exposed to volatiles attained stage III colour at about the same time as those from the control gregarious cages.

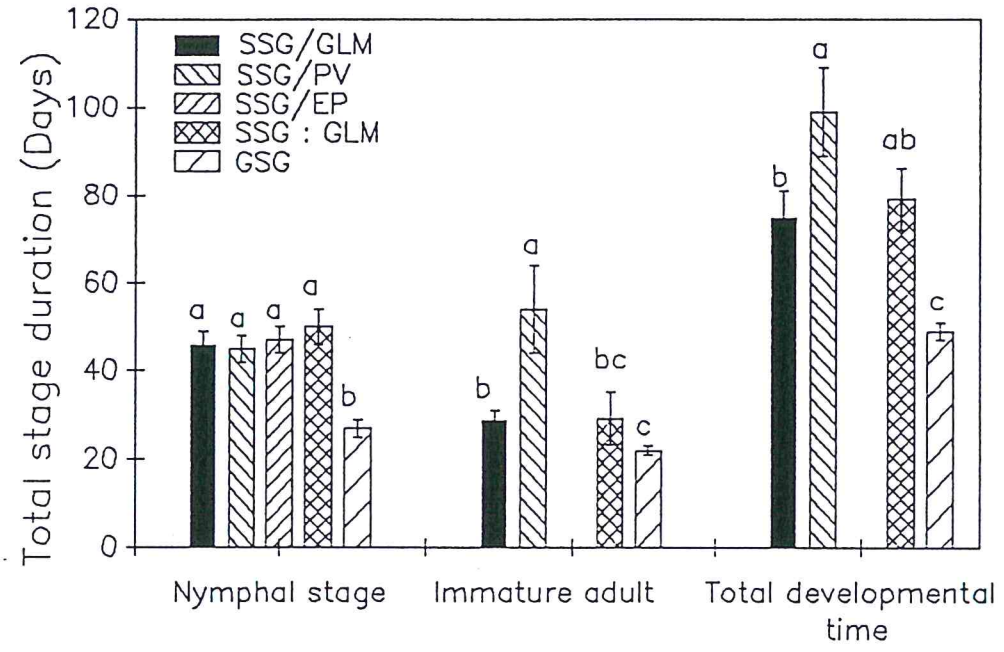

Figure 27. Stage durations of solitarious *S. gregaria*(SSG) a) reared with**[(SSG/GLM)]** or b) exposed to volatiles of **[(SSG:GLM)]** gregarious *L. migratoria*(GLM) c)reared with *P.viridipes*(PV),**[(SSG/PV)]** and d) reared with *E. plorans*(EP),**[(SSG/EP)]** compared to gregarious conspecifics from the control**[(GSG)]**.

5.5 Discussion

Primer effects of gregarious *L. migratoria* on solitarious *S. gregaria*, and vice-versa, in mixed rearing experiments, were manifested by the changes from solitarious to gregarious integumental colour, the number of instars as reflected in eye stripes, morphometrics, haemolymph pigment composition, titres of phenylacetonitrile, and the shading of the eyes. Since the experiments involved continuous mixed rearing of the two species, the shift observed in these phase characteristics may have been mediated by a combination of factors such as tactile, visual, acoustic, and olfactory cues (Chapman, 1979). From the mixed rearing experiments, it is not clear which of these are the most important in triggering and maintaining the process of phase shift. Chapman (1979) demonstrated that a stimulation by innate objects was enough to trigger the development of gregarious characters in the desert locust. Simpson (communication at the Locust Research Programme Workshop, 1994) confirmed the effects of tactile factors by exposing solitarious insects to rolling plastic balls. Visual factors have also been shown to be important in inducing phase shifts (Chapman, 1979). The lack of specificity of these cues led Chapman (1979) to comment that "anything could gregarize locusts". However, Gillett (1968) showed that visual and tactile isolation did not prevent the development of gregarious characters in locusts. It was

further shown that while sound does not play an important role, olfactory information is quite important for food seeking, synchronisation of maturation time, and oviposition (Gillett, 1968).

The experiments which involved the exposure of solitarious individuals of *S. gregaria* to volatiles of gregarious *L. migratoria* during rearing, showed the development of gregarious characteristics similar to those reared together with gregarious individuals of the latter species. Even though gregarious nymphal and mature adult pheromone production (more sensitive phase parameter) had not been monitored, the results from the exposure experiments suggest that olfactory signals from the migratory locusts are as effective as the tactile, olfactory, acoustic, and visual stimuli altogether (present in mixed rearing). They demonstrate the crucial role played by pheromones in gregarization (Gillett, 1968). This aspect needs more detailed and systematic investigations. These studies also show that, compared to gregarious or solitarious desert locusts in the controls, desert locusts reared with, or exposed to volatiles of migratory locusts, and to those of grasshoppers, showed delayed nymphal developmental time and adult maturation. Thus, only conspecific volatile emissions along with other factors are adequate for optimum locust development.

The results of rearing solitarious desert locusts with pheromone-producing and non-producing grasshoppers illustrate the key role of pheromones in locust phase

shift. When reared with the 'giant' pyrgomorphid grasshopper, *P. viridipes*, the test insects shifted to transient phase with respect to most parameters. Analysis of volatiles collected from this species has shown that *P. viridipes* produces, among others, such compounds as, guaiacol, benzaldehyde, phenol, and four acids, all of which are components of the nymphal and adult male-produced pheromonal systems of the desert locust (Torto et al., 1994; 1996). No such phase shift was observed in desert locusts reared with the grasshopper, *E. plorans*, which did not produce these compounds. Both groups of test insects, however, were abnormally affected. Those reared with *P. viridipes* changed colour and morphometrics to those of transients, produced phenylacetonitrile, but stayed immature for up to 60 days. Those that had been reared with *E. plorans* did not show any evidence of phase change, and died within 10 days after fledging to immature adult stage.

Pheromone titres of *S. gregaria* under different treatments merit a special comment. Test locusts exposed to gregarious migratory locusts at the immature adult stage produced the highest titres of phenylacetonitrile followed by those associated at the nymphal stage. Both of these groups had significantly higher titres of phenylacetonitrile than the gregarious control desert locusts. Likewise, solitarious *L. migratoria* reared with gregarious *S. gregaria* at the immature stage also produced higher titres of phenylacetinitrile than the gregarious

control migratory locusts. Similar observations were made by Deng et al. (1996) who showed that crowding of solitarious locusts produces F_0 and F_1 insects that had higher titres of phenylacetonitrile compared to those in subsequent generations. These results parallel those of Michel (1980) with reference to flight activity and migratory aptitude for a colony of crowded locusts from solitarious parents isolated for several generations. The colony used in this study has been crowded or isolated for several generations(17 generations at the time of this experiment). As Deng et al.(1996) have recommended, future studies on the relationship between phase history and specific phase characters may help throw further light on the relationship between the two.

Chemical analyses of volatiles from *L. migratoria migratorioides* confirmed the presence of the four compounds previously identified in the volatiles of 10 day-old immature and mature adults of *S. gregaria* (Torto et al., 1994). Phenylacetonitrile, the key aggregation pheromone component in *S. gregaria* (Torto et al., 1994) is present in the volatiles of nymphal (Torto unpublished) and adult *L. migratoria*. This may account, in part, for the cross-aggregation responses observed between *L. migratoria* and *S. gregaria*.

In summary, the present study has established cross primer effects in the gregarization of *S. gregaria* and *L. migratoria*. Pheromones appeared to play a major role in

the gregarization of the two locust species, but the presence of conspecific pheromones is important for optimum development of *S. gregaria*.

CHAPTER SIX

6.0 Intra-and interspecific effects of volatiles of *S. gregaria* and *L. m. migratorioides* on sexual maturation times

6.1 Introduction

Chapters (IV) and (V) described the results of strong releaser and primer effects relating to gregarization resulting from the interactions between *S. gregaria* and *L. migratoria*, and also between *S. gregaria* and two grasshoppers. In the primer effects, volatile pheromones were shown to play an important role in phase change, developmental time of nymphs, and adult duration. The effects of these volatiles from nymphs and adults of the two species on the maturation of each other's young adults are not known. It is, however, known that in *S. gregaria*, nymphal aggregation pheromone retards sexual maturation of immature adults while adult aggregation pheromone accelerates it (Norris, 1954; Mahamat et al. 1993; Assad et al., 1996), but in *L. migratoria*, sexual maturation of immature has not been adequately studied.

Loher (1990) reported that males of *L.m. migratorioides*, in the presence of mature males, copulated with females of their own age earlier (13 - 14 days after fledging) than in the presence of conspecific young adults(17 - 25 days). Thus,

pheromones may also play an important role on the timing and synchrony of sexual maturation of the migratory locust(Loher, 1990). In the desert locust, pheromones are also responsible for the change in body colour (Loher, 1990). The maturation accelerating pheromones are believed to be produced in glands in the epidermis of sexually mature (yellow) adult males (Loher, 1960). In *L. m. migratorioides* a similar pheromone is suspected to be present in the epidermal glands of both sexes (Loher, 1960). Isolation of *S. gregaria* retards maturation while in *L. m. migratorioides*, isolation accelerates it (Loher, 1960). Maturation pheromones also act interspecifically since mixed rearing of mature *S. gregaria* accelerate sexual maturation of young *L. migratoria migratorioides* (Norris, 1964). Although it has been speculated that the reverse may be true, effects of mature adults of *L. migratoria* on the maturation of young adults of *S. gregaria* have not been reported.

The main objectives of this study were to compare intra and interspecific effects of volatiles of the nymphal and adult stages of the two locust species on sexual maturation of the adult stage.

6.2 Materials and methods

Maturation experiments were run in the two chamber bioassay cages described in Chapter III.

Insects and experiments

The insects used were from the same colonies as described in Chapter III of this thesis. Due to constraints in rearing *L. migratoria* (poor hatching, and space), different insect batches were used for the different experiments. Effects of volatiles on the sexual maturation of males and females were run separately. Fifth-instar nymphs (mixed sex) or adult males were used as sources of pheromones while male and female immature adults were the recipient (test) locusts in both species. Locusts used as signal sources were placed in the top chamber, and recipient ones in the bottom chamber as described in each of the experiments in 6.2.1 and 6.2.2.

6.2.1 Effects of gregarious *L.m.migratorioides* on the sexual maturation of gregarious *S. gregaria*

These effects were studied in four different experiments as described below:

Experiment 1. **Effects of fifth-instar nymphs of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* males**

	Source	Test (Immature)	Ratio
a) Fifth-instar	*S. gregaria*	*S. gregaria*	5 : 5
b) Fifth-instar	*L. migratoria*	*S. gregaria*	5 : 5
c) Immature adult	*S. gregaria*	*S. gregaria*	5 : 5

Treatments a) and c) were controls. The parameters monitored were:

Integumental colour and copulation

The integumental colour was monitored in males following the scheme (Norris, 1954) described in Chapter III, page 59-62. Each test insect in the lower chamber was considered as a replicate. Thus, for colour, 15 - 25 insects were graded daily until at least 60% of them reached colour stage III.

Locusts which had attained colour stage III (even before the 60% reached that stage) were paired with fully mature females for six hours and the number of pairs in copulation was recorded on the particular day. The average number of days taken by locusts to start copulating was noted.

Pheromone emission

To monitor pheromone emission, locusts were randomly picked from cages, and volatile collections were done at 10-

12, 15-17, 20-22, 25-27, and 30-32 days after fledging, following the procedure described in Chapter III.

Experiment 2. **Effects of mature adult males of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* males**

	Source	Test(Immature)	Ratio
a) Mature adult	*S. gregaria*	*S. gregaria*	5 : 5
b) Mature adult	*L. migratoria*	*S. gregaria*	5 : 5
c) Immature adult	*S. gregaria*	*S. gregaria*	5 : 5

Treatments a) and c) were controls. The parameters monitored were the same as in experiment 1 described above:

Experiment 3. Effects of fifth-instar nymphs of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* females

	Source	Test(Immature)	Ratio
a) Fifth-instar	*S. gregaria*	*S. gregaria*	5 : 5
b) Fifth-instar	*L. migratoria*	*S. gregaria*	5 : 5
c) Immature adult	*S. gregaria*	*S. gregaria*	5 : 5

Where a) and c) served as control treatments. Maturation was monitored by measuring the oocyte lengths in relation to the immature adult age. Basal oocyte length was considered to be a more reliable measure of sexual maturation time as colouration in females appeared to be less discernable at early immature adult stage. Thus, test females were dissected every five days. Isolated egg masses were dipped into 70% ethanol and spread open before measurements with a Wild Heerbrugg microscope (Switzerland) with a 10 grid micrometer

adapted to a X 10 lens. Readings were converted into millimeters using the following conversion formula:

Length (mm) = (units in scope x 10) / magnification

Five females were dissected at 5-7, 10-12, 15-17, 20-22, 25-27, 30-32, and 35-37 days after fledging. For each age, 50 oocytes were measured and the mean oocyte length calculated. Insects were considered mature at the time when the insects dissected had an average oocyte length between 6-8 mm (Norris, 1954) in *S. gregaria*.

Experiment 4. **Effects of mature adult males of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* females**

Source	Test(Immature)	Ratio
a) Mature adult *S. gregaria*	*S. gregaria*	5 : 5
b) Mature adult *L. migratoria*	*S. gregaria*	5 : 5
c) Immature adult *S. gregaria*	*S. gregaria*	5 : 5

Where a) and c) served as controls. Maturation was monitored using the same parameter as in experiment 3.

6.2.2 Effects of gregarious *S. gregaria* on sexual maturation of gregarious *L. m. migratorioides*

These were similarly studied in four different experiments as described below :

Experiment 5. **Effects of fifth-instar nymphs of *S. gregaria* on the sexual maturation of immature adult *L. migratoria* males**

	Source	Test(Immature)	Ratio
a)	Fifth-instar *L. migratoria*	*L. migratoria*	5 : 5
b)	Fifth-instar *S. gregaria*	*L. migratoria*	5 : 5
c)	Immature adult *L. migratoria*	*L. migratoria*	5 : 5

Where combinations a) and c) were controls. The parameters monitored were:

Integumental colour and copulation

The integumental colour was monitored in males following Norris (1954) scheme described in Chapter III. In addition, the appearance of the yellow colour on the frons was monitored. Each test insect in the lower chamber was considered as a replicate. Thus, for colour, 15 - 25 insects were graded daily.

Locusts that were sexually mature were also paired with fully mature females for six hours and the number of pairs in copulation was recorded on the particular day, and the average number of days taken by locusts to start copulating was noted.

Pheromone emission

As described in Section 6.2.1, locusts were randomly picked from cages to monitor pheromone emissions, and volatile

collections were done at 10-12, 15-17, 20-22, 25-27, and 30-32 days after fledging, following the procedure described in Chapter III.

Experiment 6. **Effects of mature adult males of *S. gregaria* on the sexual maturation of immature adult *L. migratoria* males**

	Source	Test(Immature)	Ratio
a)	Mature adult *L. migratoria*	*L. migratoria*	5 : 5
b)	Mature adult *S. gregaria*	*L. migratoria*	5 : 5
c)	Immature adult *L. migratoria*	*L. migratoria*	5 : 5

Where treatments a) and c) served as controls. The parameters monitored were the same as described in experiment 1 of this section.

Experiment 7. **Effects of fifth-instar nymphs of *S. gregaria* on sexual maturation of immature adult *L. migratoria* females**

	Source	Test(Immature)	Ratio
a)	Fifth-instar *L. migratoria*	*L. migratoria*	5 : 5
b)	Fifth-instar *S. gregaria*	*L. migratoria*	5 : 5
c)	Immature adult *L. migratoria*	*L. migratoria*	5 : 5

where a) and c) were control treatment combinations. The parameter monitored was the length of basal oocyte as described in Section 6.2.1. Test locusts were considered mature when their basal oocyte lengths were between 4 - 6.5 mm (Descamps and Wintrebert, 1961; Highnam and Haskell, 1964).

Experiment 8. **Effects of mature adult males of *S. gregaria* on the sexual maturation of immature adult female *L. migratoria* females**

	Source	Test(Immature adult)	Ratio
a) Mature adult	*L.migratoria*	*L. migratoria*	5 : 5
b) Mature adult	*S. gregaria*	*L. migratoria*	5 : 5
c) Immature adult	*L. migratoria*	*L. migratoria*	5 : 5

Combinations a) and c) were controls. Maturation was monitored using the same parameter as in experiment 7.

6.3. Data analyses

Data were analyzed using SAS (1988). Square root or log (x+1) transformations were used when necessary and means were separated after ANOVA by LSD test.

6.4 Results

6.4.1 Effects of gregarious *L.m. migratorioides* on sexual maturation of gregarious *S. gregaria*

Experiment 1. **Effects of fifth-instar nymphs of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* males**

Young adults of *S. gregaria* exposed to volatiles of fifth-instar nymphs of *L. migratoria* changed colour and mated at about the same time as control conspecific immature adults, but significantly earlier than those exposed to conspecific fifth-instar nymphal volatiles (fig. 28).

Males of *S. gregaria* exposed to volatile emissions of fifth-instar nymphs of *L. migratoria* produced peak pheromone titres by 15-17 days after fledging, that is, about 10 days earlier than those exposed to volatiles of conspecific immature adults and fifth-instar nymphs (Fig. 29).

Experiment 2. **Effects of mature adult males of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* males**

Young adults of *S. gregaria* exposed to volatiles of mature adults of *L. migratoria* changed to colour stage III significantly ($P \leq 0.05$) earlier than control conspecific immature adults, but at about the same time as those exposed to volatiles of conspecific mature adults

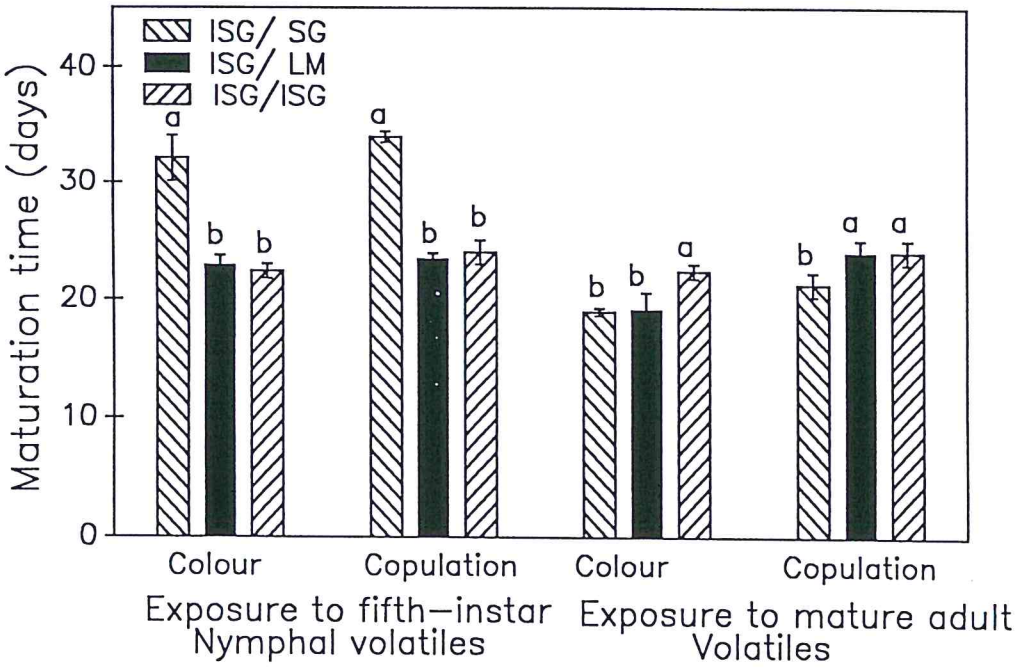

Figure 28. Time (days) taken by maturing adult males of *S. gregaria*(ISG) to attain stage colour III when exposed to volatiles of live conspecific fifth-instar nymphs and mature adults**[(ISG/SG)]**; conspecific immature adults**[(ISG/ISG)]**; and to those of similar stages of *L. migratoria***[(ISG/LM)]**.

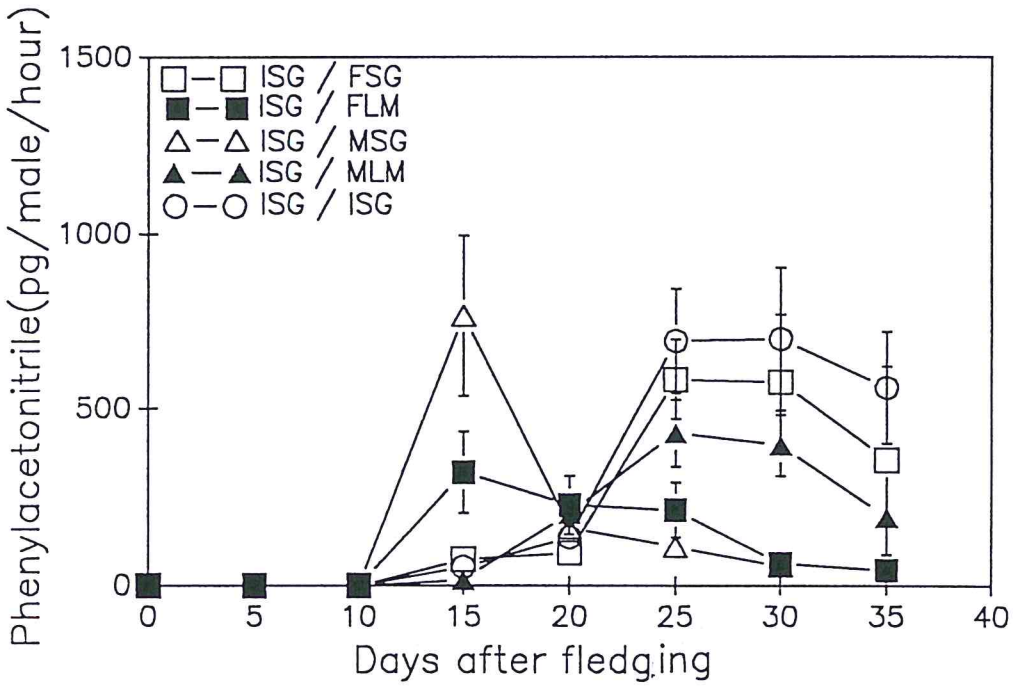

Figure 29. Amounts(pg) of phenylacetonitrile produced by maturing males of *S. gregaria*(ISG) exposed to volatiles from live conspecific fifth-instar nymphs(FSG),**[(ISG/FSG)]**; immature adults**[(ISG/ISG)]** and mature adults (MSG),**[(ISG/MSG)]**; and to those of fifth-instar(FLM),**[(ISG/FLM)]** and mature adults(MLM),**[(ISG/MLM)]** of *L. migratoria*.

(Fig. 28).

However, in mating experiments there was no significant difference.

Sexually immature males of *S. gregaria* exposed to volatile emissions of mature adults of *L. migratoria* had peak pheromone production at 25 days after fledging similar to conspecific immature adults. This peak was, however, delayed (10 days) compared to those exposed to volatiles of conspecific mature adults (Fig. 29).

Experiment 3. **Effects of fifth-instar nymphs of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* females**

Young adult females of *S. gregaria* exposed to volatiles of fifth-instar nymphs of *L. migratoria* matured significantly earlier (10-12 days), with respect to average oocyte length, compared to those that were exposed to volatiles of conspecific immature adult and fifth-instar nymphal controls (5-7 days) (Figs. 30).

Experiment 4. **Effects of mature adult males of *L. migratoria* on the sexual maturation of immature adult *S. gregaria* females**

Young adults of *S. gregaria* exposed to volatiles of mature adults of *L. migratoria* matured at about the same time, with respect to average oocyte length, as those exposed

to volatiles of conspecific control immature adults, but suffered significantly delayed maturation when compared to locusts that had been exposed to volatiles of conspecific mature adults (Fig. 30).

6.4.2 Effects of gregarious *S. gregaria* on the sexual maturation of gregarious *L.m. migratorioides*

Experiment 5. **Effects of fifth-instar nymphs of *S. gregaria* on the sexual maturation of immature *L. migratoria* males**

Young adult males of *L. migratoria* exposed to volatiles from fifth-instar nymphs of *S. gregaria* showed delayed changes in integumental colour compared to those exposed to volatiles of conspecific control immature adults and conspecific fifth-instar nymphs. They mated at the same time as conspecific control immature adults, but later than those exposed to conspecific fifth-instar nymphs (Fig. 31).

Immature adult males of *L. migratoria* exposed to volatiles of fifth-instar nymphs of *S. gregaria* produced peak amounts of phenylacetonitrile at 15 days after fledging, that is 5 days earlier than conspecific immature adult controls, but a delay of 5-7 days compared to those exposed to conspecific fifth-instars. Pheromone production was significantly higher than in locusts that had been exposed to volatiles of conspecific fifth-instars which produced relatively smaller amounts of the compound (Fig. 32).

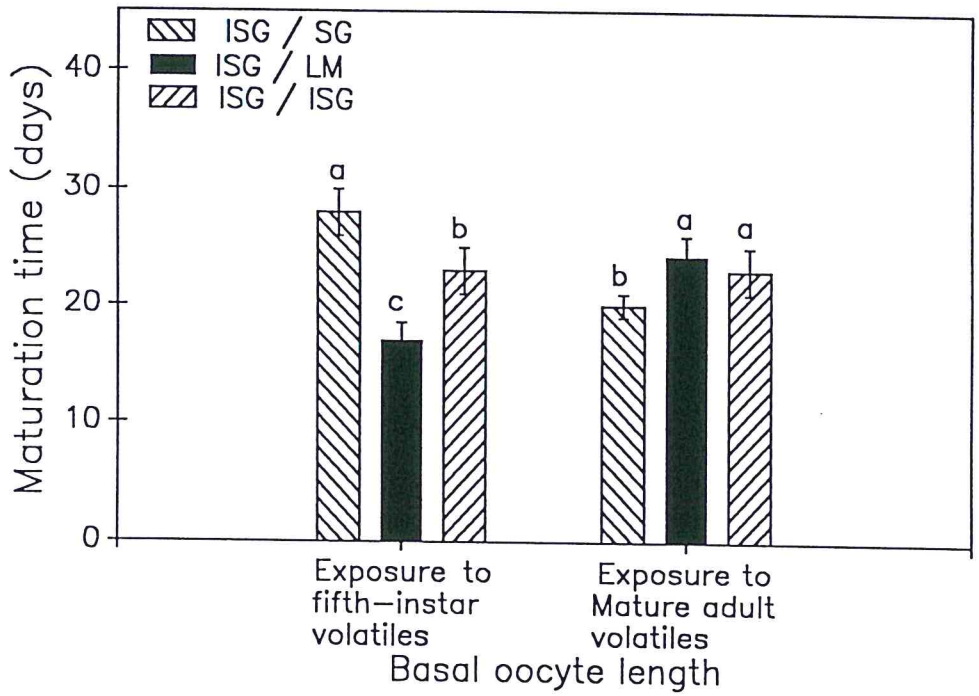

Figure 30. Time taken by maturing adult females of *S. gregaria*(ISG) to attain mature oocyte length when exposed to volatiles of conspecific(SG) fifth-instar nymphs and mature adults**[(ISG/SG)]**; conspecific immature adults**[(ISG/ISG)]**; and to those of similar stages of *L. migratoria*(LM), **[(ISG/LM)]**.

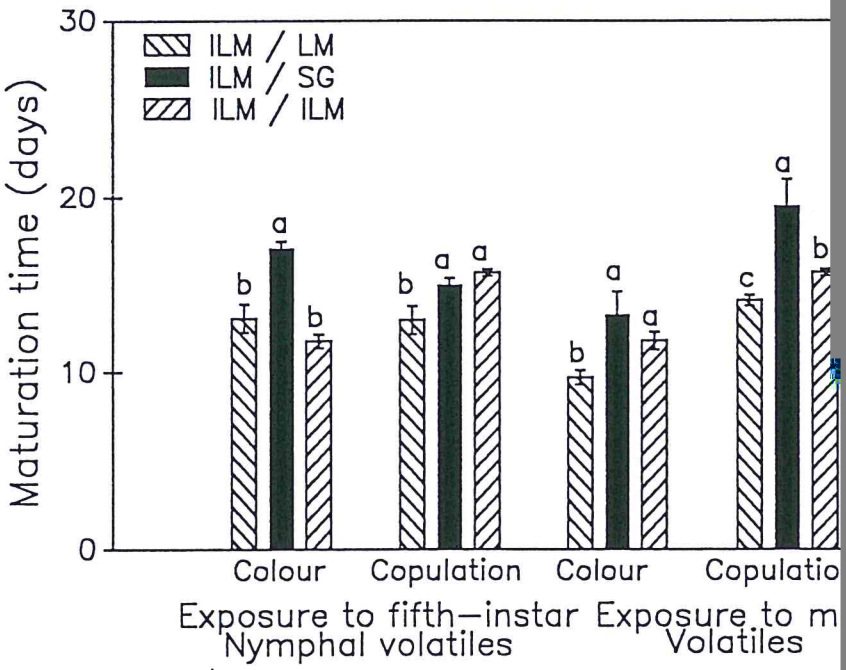

Figure 31. Time(days) taken by maturing adult males of *L. migratoria*(ILM) to attain stage colour III when expos to volatiles of live conspecific(L fifth-instar nymphs and mature adu **[(ILM/LM)]**; conspecific immature adults, **[(ILM/ILM)]**; and to those o similar stages of *S. gregaria*(SG), **[(ILM/SG)]**.

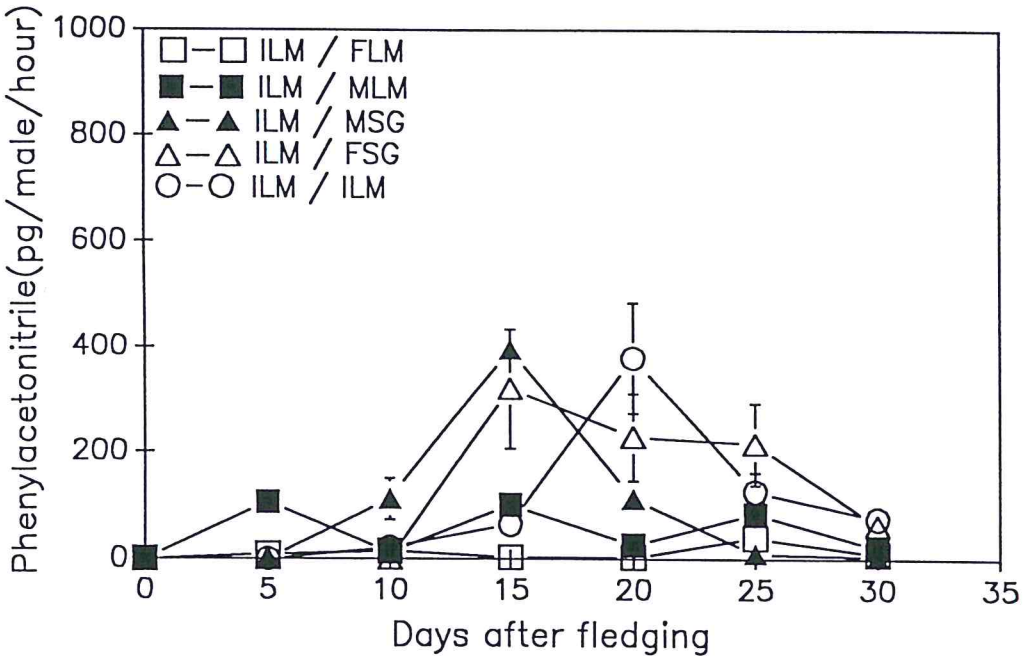

Figure 32. Amounts(pg) of phenylacetonitrile produced by maturing males of *L. migratoria*(ILM) exposed to volatiles Of live conspecific fifth-instar nymphs(FLM),**[(ILM/FLM)]** immature adults **[(ILM/ILM)]**, and mature adults(MLM) **[(ILM/MLM)]**; and to those of fifth-instar (FSG),**[(ILM/FSG)]** and mature adults(MSG), **[(ILM/MSG)]** of *S. gregaria*.

Experiment 6. Effects of mature adult males of *S. gregaria* on the sexual maturation of immature *L. migratoria* males

Young males of *L. migratoria* exposed to volatiles of mature adults of *S. gregaria* changed colour and mated later compared to those exposed to volatiles of both conspecific control immature adults, and mature adults (Fig. 31).

Immature adults of *L. migratoria* that were exposed to volatiles of mature adults of *S. gregaria* produced peak amounts of phenylacetonitrile also at 15 days after fledging which was 5 days earlier than those that were exposed to conspecific immature adult controls, but 10 days later than those exposed to volatiles of conspecific mature adults (Fig. 32).

Thus, both fifth-instar nymphs and mature adults of *S. gregaria* delayed sexual maturation of immature *L. migratoria*.

Experiment 7. Effects of fifth-instar of *S. gregaria* on the sexual maturation of immature *L. migratoria* females

Immature adult females of *L. migratoria* exposed to volatiles of fifth-instar nymphs of *S. gregaria* matured significantly earlier (5-10 days), with respect to lengths of basal oocytes, than those exposed to volatiles of conspecific immature adults, but later (5-10 days) than those exposed to volatiles of conspecific fifth-instars (Fig. 33).

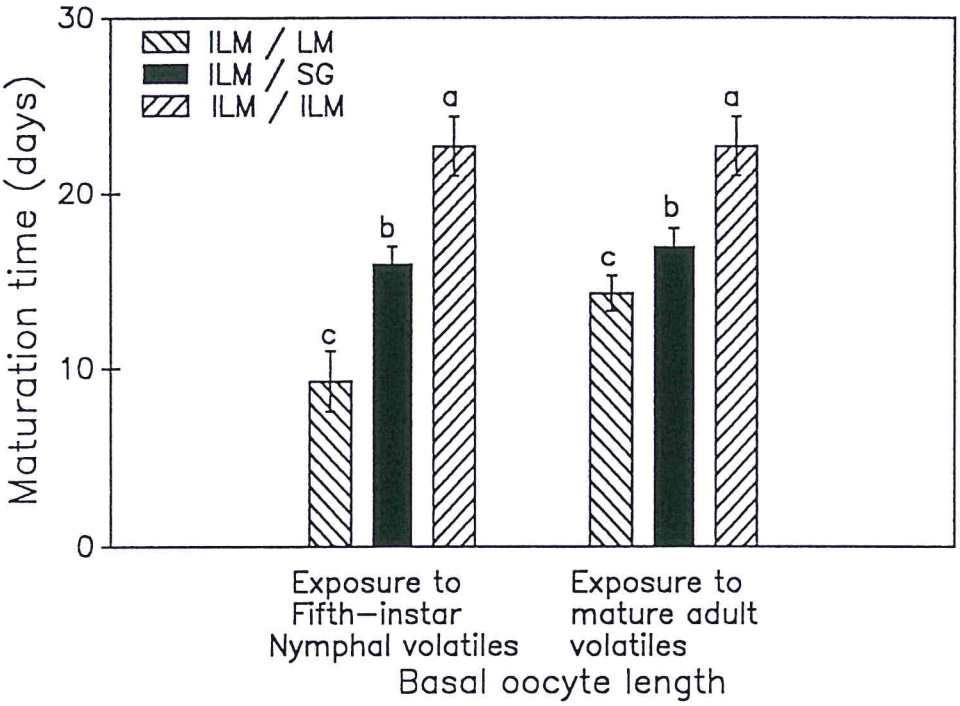

Figure 33. Time taken by maturing adult females of *L. migratoria*(ILM) to attain mature oocyte length when exposed to volatiles of conspecific(LM) fifth-instar and mature adults, **[(ILM/LM)]**; conspecific immature adults **[(ILM/ILM)]**; and to those of similar stages of *S. gregaria* (SG), **[(ILM/ISG)]**.

Experiment 8. **Effects of mature adult males of *L. migratoria* on the sexual maturation of immature *S. gregaria* females**

Immature adults of *L. migratoria* that had been exposed to volatiles of mature adults of *S. gregaria* also matured earlier than those exposed to conspecific immature adults (7-10 days) with respect to average basal oocyte length, but slightly later (3-5 days) than those exposed to conspecific mature adults (Fig. 33).

In summary, the effects of exposing the respective locusts to each other's volatiles are presented in comparison to the effects of conspecific immature adults first, then to conspecific nymphal or mature adult stages, respectively. The following are the main effects observed:

1) - fifth-instar nymphs of *L. migratoria* accelerated the sexual maturation of immature *S. gregaria*. These acceleration effects were more obvious in pheromone production and ovulation. Mature adults produced variable effects in general, ranging from neutral to acceleration, and sometimes a delay.

2) - fifth-instar nymphs and mature adults of *S. gregaria* retarded the sexual maturation of immature *L. migratoria* with fifth-instar causing more clear-cut retardation effects.

6.5 Discussion

The present results show that fifth-instar nymphal volatiles of *L. migratoria* like those of mature males of *S. gregaria* accelerate sexual maturation of immature *S. gregaria*. This is consistent with the analyses of nymphal *L. migratoria*'s volatiles which were shown to contain phenylacetonitrile (Torto unpublished) a compound also present in the volatiles of mature adults of *L. migratoria*, but in much smaller amounts compared to those in mature adults of *S. gregaria*. Phenylacetonitrile is one of maturation accelerants in maturing desert locusts (Mahamat and Hassanali, unpublished).

Fifth-instar nymphs of *S. gregaria*, in contrast to those of *L. migratoria*, retard the maturation of immature adults of both species. The effect differentiates between fifth-instar nymphal volatiles of *S. gregaria* and those of *L. migratoria*, which suggest that these stages of the two species, have different pheromonal blends.

Mature adults of *L. migratoria* did not have consistent effects on the maturation of immature *S. gregaria* whereas nymphs and mature adults of *S. gregaria* appeared to retard the maturation of immature *L. migratoria*. The cause of these effects are unknown, but it is possible that the differences in their volatile systems could again be the main one.

Mahamat et al.(1993) showed that in *S. gregaria*, aggregation pheromones play a dual role with regard to

aggregation and acceleration of sexual maturation. Hence the 'aggregation-maturation-accelerating' pheromone (Obeng-Ofori et al.,1993a; Mahamat et al., 1993; Torto et al., 1994) system of *S. gregaria*. The roles of the aggregation pheromone in the maturation and other biological aspects of *L. migratoria* must await further research. The interspecific activity of pheromones on locust maturation (Norris, 1968; Loher, 1990) are clearly shown in these experiments. In contrast to what Norris (1964) postulated, after running mixed rearing maturation experiments, that mature adults of *S. gregaria* could accelerate maturation of young adults of *L. migratoria*. Studies on the effects of exposure to volatiles on the maturation of the respective locust species have shown that mature adults of the former species in most cases delay, rather than accelerate, the sexual maturation of immature adults of the latter. This is probably due to compositional and quantitative differences in their volatiles blends such that, high amounts of the maturation accelerant (phenylacetonitrile) tend to retard maturation of the migratory locust(sort of long term dose dose effect). It was shown ealier (Chapter V, page 128) that mature male migratory locusts produced relatively little amounts of phenylacetonitrile, infering that only such amounts may be enough for aggregation and proper maturation, and that higher quantities could lead to abnormal effects such as the delay observed in maturation. It is possible that aggregation pheromones acted in both species as a maturation accelerant in

accordance to what was observed in *S. gregaria* by Mahamat et al.(unpublished). Thus, pheromones may also contribute to synchrony of maturation within and between populations of the respective species. Whitman (1990) pointed out that if stimulatory and inhibitory pheromonal effects operate in the field, the demographic consequences would be great especially in the synchronisation locust maturation and in phase transformation. It was observed in these studies that *L. migratoria* matures relatively faster than *S. gregaria*. This is an important difference between the two species which in the field suggests their separation even if they coexisted as mixed bands, or up to the immature adult stage. The desert locust and the migratory locust appear to differ in maturation such that, within similar stages, they tend to inhibit each other's sexual maturation whereas between stages they tend to either inhibit or accelerate each other's maturation. Thus the cross-effects on maturation may be affected by conditions in which they coexist. The inhibitive interactions between the two locusts were also apparent in the primer effect studies on phase shift and development. The antagonistic primer effects between *S. gregaria* and *L. migratoria* have ecological significance in that they lead to species divergence in the field, thus to separation in space and time. Therefore, these locusts may not be seen in mixed swarms, unless by accident and for a limited period.

CHAPTER SEVEN

7.1 General discussion

The results of the present study confirm that the interactions between *L. migratoria migratorioides* and *S. gregaria* observed in the field are not fortuitous. Interspecific and intraspecific assays have shown that the signals from the two locust species can elicit aggregation responses from each other, and affect each other's phase shifts, development, and maturation. An important finding of these studies is the fact that the two species share some similar components in their volatile pheromone systems. It is pertinent to compare the chemical communication system of the two species and to discuss the implications of interactions in relation to their possible biological role.

General comparisons between the two species

The present study revealed several similarities and differences in the pheromone systems of *S. gregaria* and *L. migratoria*. In both species, there was no sex differentiation in either the production or responses to nymphal aggregation pheromone. In the adult stages, the older maturing males produce the adult aggregation pheromones in both species. Also, in both species, the

young adults use their fecal volatiles and those of nymphal conspecifics to aggregate. However, there are major sexual differences in the adults of the two species. First, whereas young adults of *S. gregaria* are indifferent to the conspecific nymphal pheromone, those of *L. migratoria* are actually repelled by pheromones of conspecific nymphs. Among mature adults of *S. gregaria*, there was no evidence of a volatile sex pheromone in females. However, in *L. migratoria*, the female attracted male conspecifics but not the females, indicating the mediation of a volatile sex pheromone in the gregarious phase of this species.

Differences between the two species are also apparent in the maturation process. *L. migratoria* matures relatively faster (7-13 days) than *S. gregaria* (18-30 days). Interestingly, whereas *S. gregaria* nymphal pheromone retards the maturation of conspecific young adults, that of *L. migratoria* accelerates it. The adult *S. gregaria* pheromone accelerates the maturation of conspecific young adults. The sequential retardation by the nymphal pheromone and acceleration by that of adults ensure maturation synchrony in the adults of this species. In *L. migratoria*, the male-produced adult pheromone also accelerates the maturation of young adults albeit to a lesser extent than that emitted by conspecific nymphs. Synchrony possibly arises from the

augmentative accelerated effects of the nymphal and mature adult pheromone systems.

The present study did not include the oviposition behaviour of *L. migratoria* nor the aggregation behaviour of first to fourth-instars nymphs of this species. These are, by themselves, whole areas of research, and difficult ones. However, future behavioural studies in such aspects will help throw light on the extent of similarities in the pheromone systems mediating behaviour in the two species.

Interactions between the two locusts

The present study has shown remarkable interspecific aggregation responses to pheromone emissions of the two species at both nymphal and adult stages. In particular, the nymphs responded to one another's pheromone without any discernable specificity. Likewise the young adults, which do not produce any significant amount of the adult pheromones, also responded to each other's fecal volatiles. Among mature adults, *S. gregaria* was less responsive to volatiles of *L. migratoria*. However, the latter was less discriminatory. This suggests the existence of a specific factor in the volatile blend of *L. migratoria* not present in that of *S. gregaria*.

The existence of similarities as well as differences in the pheromone blends of the two species is also

suggested by the cross-primer gregarization and maturation effects of the two species. Solitarious individuals from both locust species gregarized when reared with gregarious groups of the other species. However, mixed rearing led to longer developmental cycles compared to rearing with conspecific gregarious locusts in both species. Thus though intra and interspecific locust pheromone systems contributed to gregarization, only conspecific pheromone leads to normal development.

Cross-maturation effects also reflect some differences in the pheromone systems of the two species. Thus, whereas the maturation of adult *S. gregaria* is retarded by conspecific nymphal pheromone, on the other hand, it is accelerated by that of *L. migratoria*. The maturation of adult *L. migratoria* is retarded by *S. gregaria* nymphal pheromone. Likewise, mature *S. gregaria* pheromone retards the maturation of young *L. migratoria*; but that of mature *L. migratoria* had no consistent effects on young adult *S. gregaria*.

Pheromone composition

The similarities or differences between locust pheromonal systems is of special interest. One of the key findings in these studies is that the two locust species share some components in their aggregation pheromone systems. However, in contrast to what was

suggested in the past by other authors (Fuzeau-Braesh, 1988; Whitman, 1990) there are important qualitative and quantitative differences between the pheromone systems of the two species. Analyses of volatiles had shown that both species produce phenylacetonitrile. It is however not produced by nymphs of *S. gregaria* while mature adult males produce it in relatively high amounts (Torto et al., 1994). In contrast to nymphal *S. gregaria*, both male and female nymphal *L. migratoria* produce it in substantial titres in their volatiles (Torto unpublished) whereas mature adult males produce it in very small amounts, nearly 100 times less than by mature male *S. gregaria*. Other aggregation compounds i.e. guaiacol, and phenol, are present in the volatiles from the respective locust species. However a detailed chemical study of *L. migratoria* needs to be conducted. The presence of substantial amounts of phenylacetonitrile in nymphal *L. migratoria* explains why fifth-instar nymphs of this species accelerated sexual maturation of immature males and females of *S. gregaria*.

Pheromone-mediated interspecific interactions: biological role

Some of the key findings described in this thesis revolve around the volatile (pheromonal) factors in these interactions. Therefore, it is pertinent to discuss briefly the importance of pheromones in locust biology

and in particular interspecific interactions that may be mediated by pheromones. Pheromone communication is probably the most important mode of communication in insects (Whitman, 1990). Many insects use pheromones for either simple communication within species or for interspecific defense, as in pyrgomorphids, against natural enemies. In most social insects e.g. termites, ants, bees, and locusts, the survival of the colony is dependent on these chemical messengers which have various functions: aggregation, dispersion, alarm, and defense. The role played by pheromones is primarily to ensure communication within species as a way of keeping populations either together or separated when necessary, and for species and mate recognition during courtship. However, in locusts, pheromones are the most important mediators of key attributes of the gregarious phase of the insect. In grasshoppers, chemical communication is less well known probably because of lack of research in this area. These insects also make extensive use of acoustics and vision besides olfactory signals within their habitats (Whitman, 1990).

Interactions between species occur when species become receptive to each other's semiochemicals, due to some ecological relationship or coevolution, such as in predator/prey, symbiotic, and mutualistic relationship (Jaffe et al., 1995). A list of compounds shared by grasshoppers and locusts was summarized by Whitman (1990). For example, phenol, guaiacol and benzaldehyde,

which are components of the male produced aggregation pheromone in *S. gregaria* (Torto et al., 1994) are also present in the defense secretions of *Romalea sp.*, a very aposematic pyrgomorphid grasshopper. They are also found in the volatiles of *P. viridipes*. Interestingly, solitary *S. gregaria* reared with *P. viridipes* turned gregarious similar to those reared with *L. migratoria*. The results on the cross reactivity of the aggregation pheromone systems of *L. migratoria* and *S. gregaria* account for the occurrence of mixed bands and mixed populations of immature adults and 5th-instar nymphs of the two locust species often observed in the field (El-Bashir and Abdel-Rahman, 1991; Johnston and Buxton, 1949; Torto, pers. comm.). I can only speculate on an adaptative value of these pheromone mediated interactions to both species. They share common ecological niches and outbreak areas. Solitarious *S. gregaria* and *L. migratoria* occupy arid to semi-arid zones where food source is scarce. They are in the constant need of finding new areas where they can develop and reproduce. To achieve this, both species have developed phase polymorphism which allows them to transform their behaviour into migratory phase with long flight characteristics. These are important for recolonizing lost (formerly colonized) habitats and the discovery of new ones. Gregarization in both species is density-dependent. In *S. gregaria* and *L. migratoria*, but

particularly in *S. gregaria*, populations occurs in very patchy vegetation with limited resources. Rapid gregarization and the resulting migratory capability of the gregarious phase are common to both species and allow the insects access to a wide profile of food plants, and thus a rapid increase in numbers. Cross-facilitation of gregarization in the early stages when the densities of each species is low can thus be adaptatively advantageous to both species.

However, the question which arises is "how long would this situation persist in the field"? In breeding areas where green vegetation and moisture are found in restricted areas such as water courses, locusts are also forced to encounter each other and interact. This also occurs during hot or cold temperatures in the habitats when insects have to seek for refuge under such plants as *Zygophyllum simplex L.* or other shrubs or straw (Descamps, 1961a; Steedman, 1988; El-Bashir and Abdel-Rahman, 1991). Nymphs are more subject to these sorts of interactions because they cannot move over long distances. Long contact between the two species, however, may lead to mutual inhibition between different stages such that development and maturation between the two species are not synchronized, leading to divergence in developmental rates. Our studies on mixed rearing have shown that *L. migratoria* slows down nymphal development of *S. gregaria*; therefore, in mixed nymphal populations the former species tends to develop faster,

fledge into immature adult stage and swarm earlier than the latter. Thus, the occurrence of mixed bands is a temporary phenomenon which may last until locusts reach the soft immature stage, not beyond. Under optimal conditions, immature *L. migratoria* matures relatively faster than immature *S. gregaria*. If immature adults of the latter are in the presence of fifth-instar nymphs of the former, their sexual maturation is accelerated; if immature adults of the former are in the presence of fifth-instar nymphs of the latter, their maturation is delayed. Thus divergence also occurs in mixed populations that may persist to the fledgling stages.

Another limiting factor to prolonged coexistence (interactions) of the two species is their food habits. *S. gregaria* is a general feeder while *L. migratoria* is restricted to graminaceous plants, thus the two are likely to diverge into different habitats even in the nymphal stage.

Whether speculations outlined here represent a valid description of real events in the field must await more detailed ecological studies of mixed locust populations.

7.2 Recommendations

The preceeding discussions suggest the following lines for future research:

1) - Studies of the same scale as those by Obeng-Ofori et al. (1993, 1994a;b), and Torto et al.(1994) need to be done for *L. migratoria, L. pardalina* and other locusts and grasshoppers, in order to have a better understanding of the possible cross influence between chemical communication systems in these species, and where they co-occur, locust phase dynamics and swarm formations;

2) - Detailed chemical studies involving the isolation and characterization of aggregation pheromones of the migratory locusts, and their similarity or differences with those of the other grasshoppers and locusts, in order to have a more comprehensive understanding of the general effects of pheromones on the ecology of acridids in general.

3) - Similar to work done in *S. gregaria* (Hansson et al., 1996; Anton and Hansson, 1996), physiological studies may be conducted to elucidate the detection of the aggregation pheromones in the central nervous system in *L. m. migratorioides*.

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APPENDICES

Appendix I. Number of *S. gregaria* (SG) or *L. migratoria* (LM) (out of 100 tested) that aggregated in treated and non treated (Blank) sides in the two choice olfactometer.

COMBINATIONS	Doses of source (LEQ)				
	3	7	10	20	40
Source / Recipient	Fifth-instar nymphs				
LM / SG	58 ± 8 a	62 ± 6 a	66 ± 7 a	74 ± 6 a	60 ± 8 a
SG / SG	66 ± 9 a	72 ± 5 a	74 ± 7 a	68 ± 7 a	68 ± 7 a
Blank / SG	36 ± 7 b	36 ± 7 b	34 ± 7 b	24 ± 3 b	36 ± 7 b
SG / LM	72 ± 6 a	78 ± 5 a	74 ± 9 a	74 ± 5 a	78 ± 7 a
LM / LM	56 ± 7 b	68 ± 5 a	77 ± 5 a	80 ± 4 a	66 ± 7 a
blank / LM	22 ± 4 c	20 ± 4 b	20 ± 7 b	16 ± 4 b	20 ± 7 b
	Mature adults				
LM / SG	52 ± 8 a	70 ± 7 a	72 ± 4 a	66 ± 7 b	62 ± 11 a
SG / SG	64 ± 7 a	76 ± 7 a	82 ± 5 a	88 ± 4 a	62 ± 7 a
Blank / SG	36 ± 9 b	22 ± 6 b	24 ± 5 b	22 ± 6 c	36 ± 10 b
SG / LM	66 ± 7 a	74 ± 6 a	78 ± 5 a	86 ± 4 a	66 ± 7 a
LM / LM	56 ± 5 a	72 ± 7 a	82 ± 6 a	76 ± 6 a	72 ± 4 a
Blank / LM	22 ± 6 b	20 ± 4 b	18 ± 5 b	8 ± 4 b	28 ± 6 b

Appendix II. Average responses (% ± SE) of nymphs and mature adults of *S. gregaria* (SGR) and *L. m. migratorioides* (LMI) to each other's volatiles in the olfactometer.

Stage	Treatment (Source / Test) combinations			
	LMI / LMI	SGR / LMI	SGR / SGR	LMI / SGR
Fifth-instar	46 ± 7 ab	56 ± 8 a	40 ± 10 ab	31 ± 10 b
Mature adults	53 ± 8 a	52 ± 8 a	37 ± 10 b	55 ± 8 a

In lines and columns, means followed by the same letter are not significantly different ($P \leq 0.05$ LSD test). Treatments are compared horizontally, and stages vertically (80 replicates of 10 min each).

Appendix III. Dose responses of nymphal and mature adult locusts of species *S. gregaria* and *L. migratoria* in a two choice olfactometer.

Locust Stage	Doses (LEQ)				
	3	7	10	20	40
Nymphs	33 ± 10 bc	43 ± 7 ab	48 ± 9 ab	54 ± 7 a	39 ± 10 ab
Mature adults	31 ± 10 c	51 ± 8 b	64 ± 8 ab	66 ± 7 a	36 ± 10 c

Within each line and column, means followed by the same letter are not significantly different ($P \leq 0.05$ LSD test).

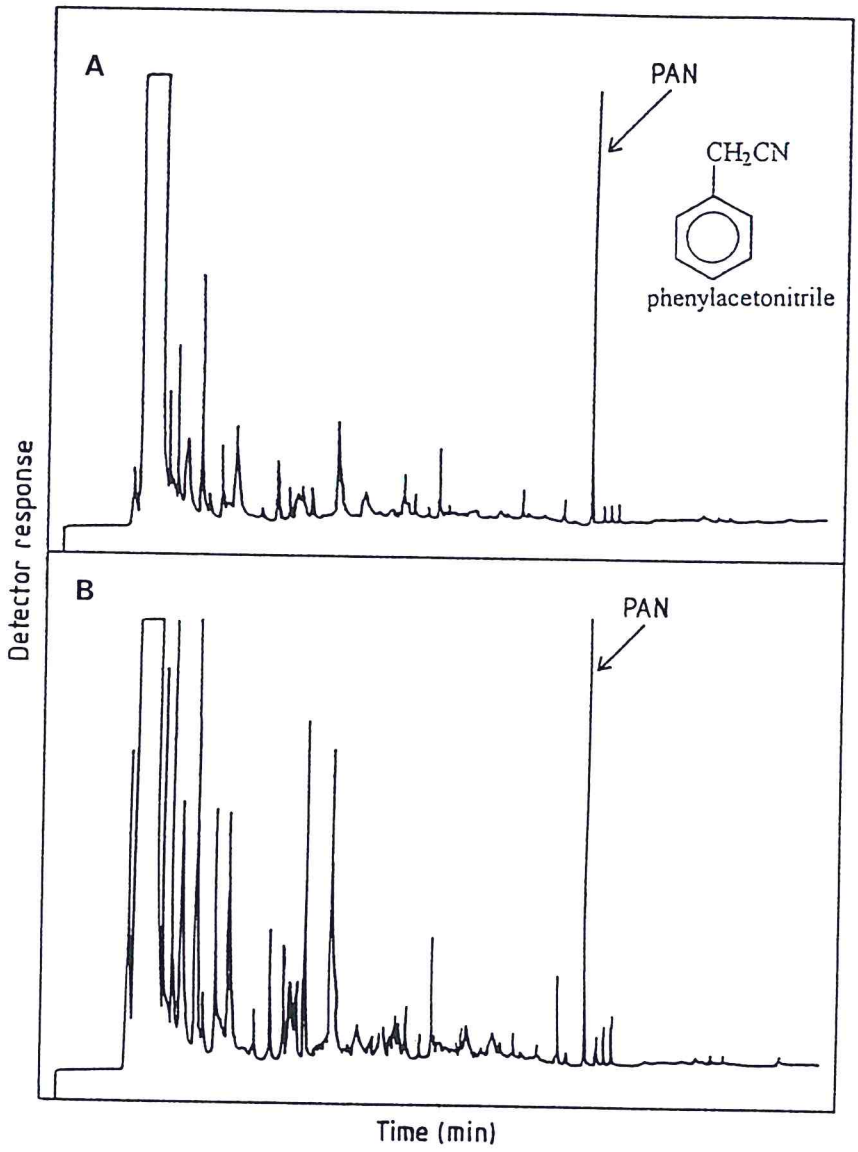
Appendix IV. Densities of crowded *L. m. migratorioides* (LM) in relation to the gregarization of each isolated *S. gregaria* (SG) under study.

NYMPHAL STAGE				ADULT STAGE			
No	Sexe	Density of LM	Density of test SG	Density of adult LM	Eyes of test SGR		Phase of test SG
					stripes	shading	
1	Female	273	5	154	7	-	Transiens
2*	Male	117	5	117	6	3	Transiens
3	Female	71	5	66	7	-	"
4	Female	120	5	33	7	-	"
5	Male	122	5	57	7	3	"
6	Male	200	5	185	6	5	gregarious
7	Male	400	5	244	6	3	"
8	Female	200	-	200	7	-	
9	Male	131	5	108	6	1	transiens
10	Male	200	5	154	7	3	"
11	Male	400	5	200	6	3	gregarious
12	Male	80	2	120	7	1	Solitary
13	Male	80	3.5	120	7	1	Solitary
14	Male	80	3.5	80	7	1	Solitary
15	Male	80	2	80	7	1	Solitary
16	Male	80	5	80	6	3	gregarious
17	Female	80	5	80	6	3	"
18	Female	80	5	80	6	3	"
19	Female	80	2	80	7	1	Solitary
Mean ± SE		152 ± 24	4 ± 0.3	118 ± 13	6.6 ± .1	2.2 ± 0.4	Transiens

Appendix V. Developmental time of *S. gregaria* (SG) reared with *L. migratoria migratorioides* (LM), *P. viridipes* (PV) and *E. plorans* (EP), exposed (:) to volatiles of *L. migratoria*, and in controls.

Trts	Nymphal instars							Im. ad	Total
S/C	1	2	3	4	5	6	Total		
SG/LM	6 ± 1c	5 ± 1ab	8 ± 1ab	8 ± 1ab	9 ± 2b	10 ± 2a	46 ± 3a	29 ± 2b	75 ± 6.4 b
SG/PV	15 ± 0a	4 ± 1bc	7 ± 1b	9 ± 1a	9 ± 1b	11 ± 2a	45 ± 3a	54 ± 10a	99 ± 10 a*
SG/EP	5 ± 0c	6 ± 1a	9 ± 0ab	6 ± 2b	12 ± 1a	9 ± 1a	47 ± 3a	8 ± 1d	55 ± 3 c**
SG:LM	10 ± 1b	6 ± 1a	10 ± 1a	7 ± 2ab	8 ± 2b	9 ± 2a	50 ± 4a	29 ± 6c	79 ± 8 b
GLM	6 ± 1c	4 ± 1bc	7 ± 1b	6 ± 1b	10 ± 1b	.	33 ± 2b	12 ± 1d	45 ± 3 c
GSG	5 ± 1cd	5 ± 1ab	5 ± 1c	5 ± 1b	8 ± 1b	.	27 ± 2c	22 ± 1c	49 ± 2 c
SSG	4 ± 0d	5 ± 1ab	4 ± 1c	3 ± 1c	7 ± 1b	12 ± 1a	32 ± 1b	.	.
SLM	4 ± 0d	3 ± 0c	4 ± 0c	8 ± 2ab	12 ± 1a	.	31 ± 1b	.	.

SSG: solitary control; Trts = treatment combinations; S: solitarious test locust; / : over. C : gregarious source. In columns, means followed by the same letter are not significantly different ($P \leq 0.05$, LSD-test). Im. ad = immature adult; * : 50% reached maturation colour in 34 ± 3 days. The other 50% never did by the end of the experiment. ** : died 8 days after fledging



Appendix VI. Chromatograms showing phenylacetonitrile (PAN) produced by gregarious fifth- instar nymphal male (A) and female (B) *L. migratoria* 3 - 4 days after molt (50m, 0.2mm ID Carbowax column).

Appendix VII : Compounds identified from the volatiles of adult *P. viridipes* Staal, 1873 used in the studies of primer effects of pheromones (+ means that the compound is found in that species).

Compound	*P. viridipes*	*L. migratoria*	*S. gregaria*
Benzaldehyde	+	+	+
Pentanoic acid	+		
Hexanoic acid	+		
Guaiacol	+	+	+
Heptanoic acid	+		
Phenol	+	+	+
Octanoic acid	+		
Nonanoic acid	+		
Decanoic acid	+		

Appendix VIII. Percent (%) of *S. gregaria* showing phase change after rearing with different stages of *L. migratoria*

Parameters	Nymph/Nymph			Immat. /Immat.			Immat./Nymph			Nymph : Nymph		
	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.
Body colour	75	57	69	100	32	89	80	90	75	100	100	100
Morphometrics	27	38	33	.	.	.	.	.	.	50	50	50
Haemolymph	80	100	81	.	.	.	.	.	.	.	.	.
PAN *	*	*	*	*	*	*	*	*	*	.	.	.
Eye stripes	44	6	50	.	.	.	.	.	.	50	0	25
Eye shading	100	13	56	80	75	78	100	100	100	.	.	.

/ = mixed rearing; : = exposed to volatiles of *L.m. migratorioides*. M = males F = Females. PAN = phenylacetonitrile. * : males were grouped for pheromone collection; they produced the pheromone; . : parameter not monitored for this experiment. Tot. : total immat. = immature adult

Appendix IX. Mean basal oocyte lengths in maturing females of *S. gregaria* (ISG) and *L. m. migratorioides* exposed to volatiles of fifth-instar nymphs (FSG or FLM), conspecific immature, and mature adult (MSG or MLM) volatiles.

Combinations	Days after fledging (range of ± 1 day)					
Source / Test	6	11	16	21	26	31
ISG / ISG	2.4 ± 0.2	4.1 ± 0.3	4.9 ± 0.2	5.3 ± 0.3	5.6 ± 0.1	.
MSG / ILM	1.7 ± 0.6	2.4 ± 0.3	5.0 ± 0.5	4.3 ± 0.3	4.8 ± 0.5	3.0 ± 0.2
MLM / ISG	1.8 ± 0.2	2.8 ± 0.5	3.8 ± 0.9	5.0 ± 0.9	3.2 ± 0.5	.
FSG / ISG	.	2.7 ± 0.9	4.9 ± 0.8	5.9 ± 0.4	5.6 ± 1.0	4.1 ± 0.8
FSG / ILM	1.0 ± .01	1.8 ± 0.3	5.5 ± 0.0	4.2 ± 0.2	4.9 ± 0.5	3.9 ± 0.5
FLM / ISG	.	2.2 ± 1.0	6.5 ± 0.3	5.0 ± 0.6	6.0 ± 0.8	5.6 ± 0.1
MSG / ISG	.	2.0 ± 0.5	5.2 ± 0.5	6.4 ± 0.7	3.3 ± 0.7	5.4 ± 0.6
MLM / ILM	1.9 ± 0.1	4.9 ± 0.3	5.5 ± 0.0	4.4 ± 0.4	4.7 ± 0.2	4.3 ± 0.1
ILM / ILM	1.0 ± 0.4	3.2 ± 0.0	4.1 ± 0.1	4.9 ± 0.8	4.9 ± 0.2	3.6 ± 0.1
ILM / ILM	2.7 ± 0.8	5.8 ± 0.7	3.0 ± 0.6	4.9 ± 0.5	5.1 ± .04	5.5 ± 0.1

A total of 25 female locusts in clusters (blocks) of 5 locusts were tested for each experiment or combinations.

Appendix X. Summarized effects of *L. migratoria* and *S. gregaria* on each other's sexual maturation with respect to integumental colour, copulation, oocyte length, using two conspecific effects as references.

Combinations		Parameters						Effects	
Source	Test	Colour						On ISG	On ILM
		ISG	FSG	MSG	ILM	FLM	MLM		
FLM	ISG	±	+	.	+	±	+	Acceleration	
FSG	ILM	.	.	.	−	−	.		Delay
MLM	ISG	−	.	±	±	+	−	intermediate	
MSG	ILM	.	.	.	−	.	±		Delay
Copulation									
FLM	ISG	±	+	.	+	±	+	Accelerate	
FSG	ILM	.	.	.	±	−	.		delay
MLM	ISG	−	.	−	±	+	−	Delay	
MSG	ILM	.	.	.	−	.	−		Delay
Pheromone									
FLM	ISG	+	±	.	+	±	+	Accelerate	
FSG	ILM	.	.	.	+	−	.		Intermediate
MLM	ISG	+	.	±	±	+	−	Accelerate	
MSG	ILM	.	.	.	+	.	±		Accelerate
Oocyte lengths									
FLM	ISG	+	+	.	+	±	+	Accelerate	
FSG	ILM	.	.	.	+	−	.		Intermediate
MLM	ISG	±	.	−	±	+	−	Delay	
MSG	ILM	.	.	.	+	.	±		Accelerate

FLM : fifth-instar *L. migratoria*; FSG : fifth-instar *S. gregaria* MLM : mature adults *L. migratoria*; MSG : mature adult *S. gregaria*.