Advances, controversies and consensus in locust phase polyphenism research

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Abstract

The present paper arose from a symposium at the 9th International Conference of the Orthopterists' Society held in Canmore, Canada, from 14-19th August 2005. Most of the major groups working on locust phase polyphenism were in attendance (Table 1), offering the opportunity to review the rapid progress that has occurred in this field over recent years. To maintain momentum in this research, areas where results from different groups are at odds were debated, and ways to reconcile such discrepancies proposed. The symposium also provided researchers with a forum to consider how to coordinate core facilities and resources across laboratories, to make best use of national and international funding opportunities. Participants presented their results spanning a range of aspects of phase polyphenism research: molecular analyses, physiology, ecology and phylogenetic reconstruction of the evolutionary history of phase change. The symposium was followed by a detailed discussion session, attended by members of the audience as well as the symposium speakers. The present paper provides a synopsis of that discussion and is structured according to the major issues considered. Unless otherwise stated, the paper concerns the desert locust, *Schistocerca gregaria*, which has been the subject of most of the recent research.

Introduction

Phase polyphenism is a form of density-dependent phenotypic plasticity in which the same genotype may express graded changes between two extreme forms, the cryptic phase *solitaria* and the aggregating, highly mobile phase *gregaria* (Pener 1991). Given certain environmental conditions, solitarious-phase locusts are brought together, against their predisposition actively to avoid each other, and experience crowding at locally high population densities. In particular, the distribution and abundance of resources in the habitat are critical, with clumped resource distributions favoring congregation of local populations (Collett *et al.* 1998; Despland *et al.* 2000; Despland & Simpson 2000a,b; Despland *et al.* 2004; Babah & Sword 2004). Under these conditions locusts will be forced into close contact, triggering a rapid behavioral phase change, which results in them beginning actively to aggregate, rather than being repelled by each other. This is a key event as it sets up a positive feedback loop, driving further crowding and the production of larger and larger aggregations.

Continued crowding provides the stimuli for the expression of additional phase characteristics, which include changes in coloration, morphometry, anatomy, food selection, nutritional physiology, reproductive physiology, metabolism, neurophysiology, endocrine physiology, molecular biology, immune responses, longevity and pheromone production (reviewed by Pener 1991 and Pener & Yerulshalmi 1998; see also Simpson *et al.* 2002, Wilson *et al.* 2002, Ferenz & Seidelmann 2003, Kang *et al.* 2004, Hassanali *et al.* 2005) (Fig. 1).

Phase change can occur within the life of an individual, but also accumulates epigenetically across generations through a water-soluble factor added to the egg foam by the mother in response to recent crowding (Islam *et al.* 1994a,b; Bouaïchi *et al.* 1995; McCaffery *et al.* 1998; Hägele *et al.* 2000).

Tactile contact, particularly of the hind femur, has recently been identified as a major stimulus triggering behavioral phase change (Hägele & Simpson 2000, Simpson *et al.* 2001), and the underlying neural and neurochemical mechanisms of phase change are being teased apart (Rogers *et al.* 2003, 2004). The combination of visual and olfactory stimuli is also behaviorally gregarizing (Roessingh *et al.* 1998, Lester *et al.* 2005), but neither is effective when presented singly, and the specific natures of the behaviorally-gregarizing visual and olfactory stimuli provided by other locusts are not known.

The stimuli evoking other phase characteristics are poorly understood. Changes in features such as color pattern in nymphs and morphometry, occur more slowly than behavioral gregarization and need not be evoked by the same stimuli, nor involve the same physiological mechanisms as behavioral phase change. For example, the smell alone of other locusts induces the development of black patterning in solitarious nymphs, yet it does not cause the production of yellow-background coloration or behavioral phase change (Lester *et al.* 2005). The combination of the sight and smell of other locusts is behaviorally gregarizing, but still fails to evoke yellowing, which requires rearing among a crowd of conspecific nymphs, presumably indicating the presence of a contact chemical cue (Lester *et al.* 2005).

The production of nymphal black patterning is controlled by the neuropeptide [His^7]-corazonin, also termed dark-color-inducing neurohormone or dark pigmentotropin (Tawfik *et al.* 1999; Tanaka 2000, 2001). [His^7]-corazonin also changes morphometry towards the gregarious condition, but has no effect on behavioral phase state (Tanaka *et al.* 2002, Hoste *et al.* 2002).

Much effort has been devoted to the possible roles of ecdysteroids and juvenile hormone in phase change, but the prevailing view is that these are not primary controlling agents of gregarization, either within a generation (Pener *et al.* 1992, Applebaum *et al.* 1997, Pener & Yerushalmi 1998) or between generations (Hägele *et al.* 2004). In a recent review, Hartfelder & Emlen (2005), restated the view that juvenile hormone is involved in gregarization. Unfortunately,

Table 1. Participants in the "Advances and Controversies in Locust Phase Polyphenism Research" plenary symposium held at the 9th International Conference of the Orthopterists' Society in Canmore, Canada, 14-19 August 2005. An asterisk indicates the presenter of a multiple-author presentation.

Speaker	Presentation title
Meir Paul Pener	Volatile pheromones, contact pheromones, mechanical touching and visual cues as signals in locust phase polyphenism: state of the art.
Arnold De Loof*, Gert Simonet, Ilse Claeys, Roger Huybrechts, Jozef Vanden Broeck	Molecular markers of phase transition in locusts.
Stephen M. Rogers*, Thomas Matheson, Michael Anstey, Stephen J. Simpson	Early induction of behavioral phase change in the desert locust.
Thomas Matheson*, Stephen M. Rogers, Holger G. Krapp	Changes in synaptic strength accompany phase change in locusts.
Seiji Tanaka	Corazonin and phase polyphenism in locusts.
Ahmed Hassanali*, Magzoub Bashir, Peter Njagi, Sidi ould Eli	The role of semiochemicals in desert locust gregarization: its genesis, spread, and sustenance.
Hans-Joerg Ferenz*, Karsten Seidelmann	Locust volatiles: pheromones for every purpose?
Karsten Seidelmann	Unravelling the function of phenylacetonitrile: courtship inhibition or aggregation?
Emma Despland*, Stephen J. Simpson	Locust food choices reflect density-dependent antipredator strategies.
Hojun Song	Phylogenetic perspectives on the evolution of locust phase polyphenism.
Gregory A. Sword	Genotype versus the environment in locust swarm formation: how important is phase polyphenism?

this was not based on new research, but earlier claims of Dorn *et al.* (2000) that did not take account of substantial contrary evidence. There are various other hormonal differences between the phases, notably in various brain-derived peptides (*e.g.*, Ayali *et al.* 1996a,b; Wedekind-Hirschberger *et al.* 1999; Clynen *et al.* 2001; Rahman *et al.* 2002), but none of these has yet been shown to be causal in phase change.

A recent study comparing relative amounts of neurotransmitters and neuromodulators in the central nervous systems of solitarious and gregarious locusts as they underwent phase transitions, underlines the complexity of physiological phase differences (Rogers *et al*. 2004). Of 13 analysed substances, 11 differed between long-term solitarious and gregarious locusts, including important excitatory (glutamate, acetylcholine) and inhibitory (GABA) neurotransmitters, as well as the neuromodulators/neurohormones dopamine and serotonin. Of all these substances however, only serotonin underwent a substantial increase (4 to 5 × solitarious values) during the critical 4-h period during which behavioral gregarization is established. Thus, of all these substances only serotonin has even the possibility of being an enabling/causal agent in a phase transition, and this is the focus of ongoing analysis.

Why study locust phase polyphenism?

Even if locusts were not a major pest, there are compelling reasons to analyze phase polyphenism. Phase change is central to the biology of locusts and presents itself as a model system with which to analyse both the causes and consequences of phenotypic plasticity. At the proximal level there have been recent studies on how the properties of identified neurones in the central nervous system differ between solitarious and gregarious locusts and how these may underlie differences in behavior (Fuchs *et al*. 2003, Matheson *et al*. 2004). For example, an identified visual interneurone, the descending contralateral movement detector (DCMD), responds most strongly to objects on a collision course with the locust. This neurone habituates far less readily, following repeated stimulation, in gregarious locusts than in solitarious individuals, presumably an adaptation to living in a visual environment dominated by the presence of other locusts (Matheson *et al*. 2004). As a model with which to analyse how changes in the central nervous system underlie behavioral modification, phase change in locusts not only offers a plethora of behavioral differences in a system unhampered by phylogenetic effects, but the added advantage that these differences are fully reversible over a number of time scales.

Moving from proximal and mechanistic differences, phase change offers itself as an ideal model to analyse: *e.g.*: epigenetic inheritance and maternal influences on offspring; the behavioral, ecological and evolutionary consequences of group, as opposed to solitary, living; and the evolution of aposematic coloration, as detailed below. Finally, locusts remain a major pest in several regions of the world, and periodic plagues can still have devastating consequences, as was distressingly apparent during the last major desert locust outbreak in Northern Africa in 2003-2004.

What is the adaptive significance of phase polyphenism in locusts?

While it is clear that different phase traits are controlled by different, overlapping sets of stimuli and can involve different underlying physiological mechanisms, recent studies suggest that at least some desert locust phase traits are functioning in concert as part of an antipredator strategy (Sword 1999, 2001, 2002; Sword & Simpson 2000; Sword *et al.* 2000; Despland & Simpson 2005a,b). The gregarious black and yellow color pattern of desert locust nymphs has been shown to function as warning coloration, signalling to potential predators that locusts have been feeding on toxicity-conferring plants (Sword *et al.* 2000). This has been further supported by recent studies showing that newly crowded solitarious-phase nymphs shift, from avoiding to preferentially ingesting, foods containing plant secondary compounds that are

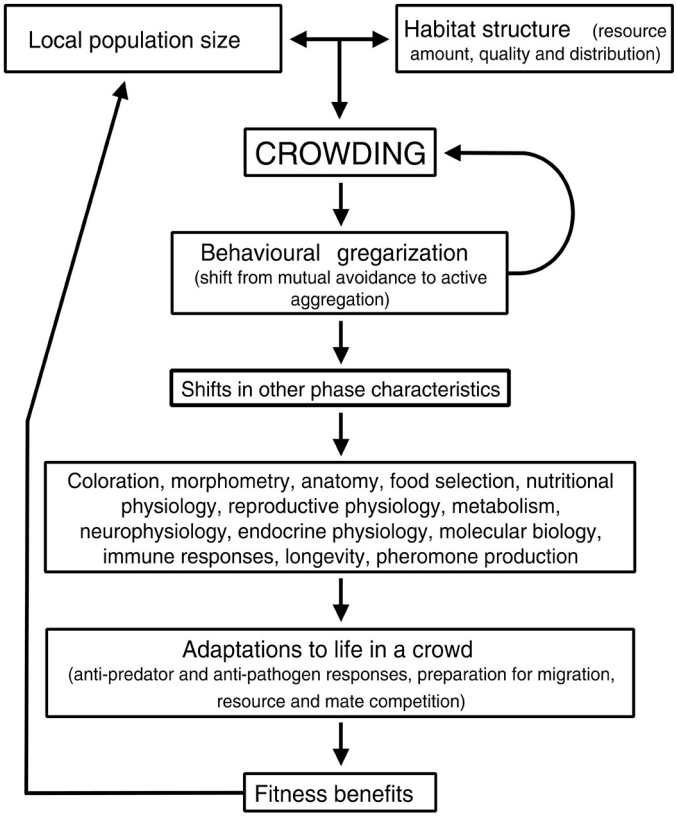

Fig. 1. Summary diagram depicting effects of local ecology and crowding on the expression of phase polyphenism in behavior and other traits.

powerful vertebrate toxins (Despland & Simpson 2005a,b). This rapidly induced change in feeding behavior serves to protect cryptically colored solitarious-phase nymphs against predators during the vulnerable period when they are behaviorally gregarious (and thus more apparent to predators), but not yet warningly colored. Only when desert locust nymphs become conspicuously colored as well as chemically protected, do they possess the full suite of characteristics in which warning coloration acts as an antipredator strategy.

A reduction in *per capita* predation rate resulting from the combination of gut content-mediated toxicity and the expression of warning coloration, will contribute to increases in local population density and band formation (Sword *et al.* 2000) (Fig. 1). Predation rates will be reduced further as bands increase in size, both through the diluting effect of numbers and also the likely amplification of the warning signal in larger groups (Gamberale & Tullberg 1998, Krause & Ruxton 2002). The protection from predators afforded to insects by migratory band formation was recently demonstrated in the migratory band-forming Mormon cricket, *Anabrus simplex* (Tettigoniidae). Using radiotelemetric methods, Sword *et al.* (2005) showed that individuals in migratory bands are much less likely to be killed by predators than insects not in the band.

Thus, in locusts the expression of a suite of density-dependent changes, functioning together as an antipredator strategy, can influence local population dynamics and lead to migratory band formation. Migratory bands themselves then also function as an antipredator strategy and likely play an important role in local population dynamics by further reducing predation on band members.

How does the warning coloration hypothesis apply to other locust species? *Locusta migratoria* nymphs, for example, express dark, conspicuously colored gregarious-phase phenotypes, yet there is no evidence to date suggesting that these color patterns function as a visual warning signal to predators. There are a number of potential explanations for this lack of evidence. One is simply that the requisite experiments to demonstrate aposematism have not yet been carried out (see Sword *et al.* 2000 for an explanation of why aposematism had been overlooked for so long in *S. gregaria*). *L. migratoria* feeds primarily on grasses, a group not generally considered to be rich in noxious secondary compounds. However, some grasses such as *Cynodon dactylon* are cyanogenic and may be candidates for conferring toxicity to predators (Wilson 2000). In addition, Whitman (1990) suggests that *L. migratoria* possess an eversible pronotal gland, capable of emitting an odorous secretion. The effect of host plant use on the secretion composition has not been addressed and its antipredator function remains unknown (Schmidt *et al.* 1987). Conversely, it may be that *L. migratoria* nymphs are entirely palatable and that their high-density phenotypes either perform some as yet undetermined function, or are selectively-neutral by-products of another adaptive density-dependent response, such as density-dependent prophylaxis (Sword 2002, Wilson *et al.* 2002).

It should not be implied that all phase characteristics serve an antipredator function, although it seems apparent that they are all adaptations to the demands of living either alone or in a crowd. Up-regulated immune responses in gregarious locusts protect against higher rates of pathogen transmission at high density (Wilson *et al.* 2002). Changes in nutrient balancing strategies, metabolism and lipid deposition, reflect preparation for migratory behavior and competition for limiting food resources (Applebaum & Heifetz 1999, Simpson *et al.* 2002). Changes in pheromonal chemistry suit a crowded lifestyle in which there is increased competition among males for access to females (Seidelmann *et al.* 2005), and differences between the phases in longevity, egg size, and number of eggs, reflect life history trade-offs between sedentary and migratory lifestyles (Uvarov 1966).

A cautionary note about the adaptive significance of phase change in locusts is warranted. Simply observing migratory bands or flying swarms should not be taken as evidence that a species expresses phase polyphenism in behavior or any other trait.

Take, for example, the Mormon cricket. This katydid forms huge migratory bands under high population density conditions that closely resemble those of locusts. In addition to differences in migratory behavior, Mormon crickets in low and high-density populations also exhibit phenotypic differences in other traits such as coloration and body size (Gwynne 2001, Lorch *et al.* 2005). These similarities led some to reasonably suspect that the Mormon cricket might express density-dependent phase polyphenism, similar to that known to occur in locusts. However, Sword (2005) failed to find any evidence for the expression of this in Mormon crickets, but found rather that their locomotion was induced simply by short-term stimuli provided by nearby conspecifics. Though this finding does not exclude other, nonbehavioral phase polyphenisms, a recent phylogeographic analysis of the Mormon cricket suggests considerable genetic divergence between migratory and nonmigratory forms, and may help to explain the observed phenotypic differences between populations (Bailey *et al.* 2005).

Nevertheless, it seems that migratory band formation has convergently evolved in Mormon crickets and locusts, via different underlying behavioral mechanisms. The expression of behavioral phase polyphenism is not a prerequisite for migratory band forma-

tion and its expression should not be assumed in species where controlled rearing experiments are lacking.

PAN–its role in locust biology

Considerable work has been published on locust semiochemicals over the past 10 y, greatly adding to our appreciation of the complexity of chemical communication in the desert locust. There is a general consensus over results in much of the literature, but a few notable exceptions have arisen (highlighted in recent reviews by Ferenz & Seidelmann (2003) and Hassanali *et al.* (2005)). Of these the most contentious is the role of phenylacetonitrile (PAN, formula $C_6H_5CH_2CN$—also known as benzyl cyanide). It is generally accepted that PAN is produced as part of the odor bouquet of mature adult males of desert locusts when they are subjected to crowding by other mature males (Luber *et al.* 1993, Torto *et al.* 1994, Deng *et al.* 1996, Seidelmann *et al.* 2000). It is not produced by nymphs, immature males, adult females of any age, by mature males that are kept alone, or by mature males kept crowded with immature males or females. The amount of PAN released by each male locust is a direct function of the number of mature males in the group, with a maximum release rate of *ca.* 200 ng male^{-1} day^{-1} (Seidelmann *et al.* 2000). The main release sites for PAN are the wings and legs, especially the forewings (Seidelmann *et al.* 2003). The glands secreting PAN have not yet been identified, but it seems likely that vacuolated epidermal cells on these body structures are responsible, though the presence of such cells in the distal part of the wings of sexually mature locusts has not yet been demonstrated.

Data from experiments conducted by the group at ICIPE, Kenya, have indicated that PAN serves to promote the aggregation of both immature and sexually mature adult (but not nymphal) locusts of both sexes (Obeng-Ofori *et al.* 1993, Torto *et al.* 1994, Hassanali *et al.* 2005). Both solitary-reared and crowd-reared adults were reported to remain within a vertical air stream containing PAN rather than in a control region of the test arena (Njagi *et al.* 1996). It was proposed that PAN acts as a 'cohesion factor', not attracting locusts over a distance, but rather causing them to stay longer in PAN-suffused areas. In short, PAN is an arrestant. Thus, adult locusts that find themselves within a crowd would tend to remain together.

The view that PAN is an aggregation (cohesion) pheromone has recently been challenged by the group working in Halle, Germany. They used a different style of olfactometer assay and published data indicating that PAN is a powerful repellent, being released by crowded, mature males to repel other rival males during courtship and mating, and to prevent unfruitful homosexual mating attempts (Seidelmann & Ferenz 2002, Ferenz & Seidelmann 2003, Seidelmann *et al.* 2005). Such a role is consistent with PAN's use by butterflies as an anti-aphrodisiac (Fatouros *et al.* 2005).

How can the 2 diametrically opposed views that PAN is an aggregation pheromone and a repellent be accommodated? There are four possible explanations, as follows.

1) PAN acts as a cohesion factor at low concentrations and as a repellent at the higher concentrations found in the close vicinity of mature males; this solution was proposed recently by Hassanali *et al.* (2005) and would imply that the concentrations tested by the ICIPE group were lower than those used in Halle. A recent paper by Seidelmann *et al.* (2005) provides the first quantification of actual PAN concentrations within a behavioral assay system, and indicates that very low concentrations do have a different behavioral effect to the repellence of higher concentrations. Locusts tended to remain in a neutral region within their arena at low concentrations. Whether this would translate into behavioral arrestment cannot be determined from the assay.

2) PAN causes cohesion among locusts at certain ages or in particular physiological states, while acting as a repellent at other times. It has been shown that PAN is not as strongly repellent to young adult males and nymphs as it is to mature males (Seidelmann *et al.* 2000), while Ignell *et al.* (2001) report that PAN is an aggregant for sexually immature adult locusts and for males in which the glands controlling sexual maturation (the *corpora allata*) have been removed. Allatectomized young females showed less aggregation than the controls, but in sexually mature females this situation was partially reversed. Contrary to earlier reports, they also found that PAN was not an aggregant for sexually mature adults. On balance, however, it is hard to see how differences in age of test insects between laboratories could account for the contrasting effects of PAN reported for sexually mature adults.

3) Features of the assay systems used and the analysis of results might account for the discrepancies. The olfactometers used by the ICIPE and Halle groups are very different. The Halle assay is not ideal for detecting arrestment, but the ICIPE assay should be able to detect repellence—insects would simply avoid the PAN-treated side of the arena and move to the control side. However, Ferenz & Seidelmann (2003) pointed out a flaw in the analysis of data from the ICIPE group's bioassay in at least one of the key papers (Torto *et al.* 1994). The basis for analysing the behavioral effect of PAN and other odorants is the calculation of an 'aggregation index' in which numbers of locusts on the control side of the arena are subtracted from those on the test side, then divided by the total number of insects tested and multiplied by 100. Positive values of this index indicate that locusts tended to remain within the test odor stream, whereas negative values would indicate repellence. In Torto *et al.* (1994) it is stated that negative values were set to zero. This would lead to values of < 30 (rather than < 0) equating to repellence, yet this was not taken into account. It is important to know in which other papers this practice was used.

4) A further possibility is that the locust cultures used by the two groups differ in their semiochemistry—either quantitatively or qualitatively. For example, using locusts from the ICIPE culture, Njagi & Torto (2002) reported that the release of propionic acid from the Comstock-Kellog glands of adult females, serves as a male attractant. Attempts to replicate this finding using locusts from the colony established in Halle have so far been unsuccessful (Seidelmann & Ferenz, pers. com.), but laboratory strain effects as a potential confound cannot be ruled out.

It is extremely important for the future of locust semiochemical research to resolve these possible experimental differences. The first priority is for both laboratories to work together to derive dose-response curves for PAN, in which actual concentrations within the arenas are measured. Individual locusts rather than groups should be tested and care taken that both arrestment behavior and repellence are detected and quantified. It would be productive to analyse rates of turning and speed of locomotion within PAN-suffused regions, to identify possible mechanisms of arrestment. Testing of each other's locusts would also be very worthwhile.

The evolution of phase polyphenism: morphology versus molecules

The expression of phase polyphenism correlates with the development of locust outbreaks and subsequent mass migration, but the cause and effect relationship between these phenomena can be

difficult to establish (Key 1950, Sword 2003). Importantly, this correlation may be weak or even absent in some locusts. For example, the Australian plague locust, *Chortoicetes terminifera*, expresses little or no obvious discernable phase changes (Uvarov 1966), but it does exhibit intense swarming (Hunter 2004). However, field observations by Clark (1949) describe what clearly appears to be behavioral phase change in nymphs. As discussed earlier, these observations highlight the need for detailed empirical analyses to be conducted in order to establish the presence or absence of phase polyphenism in specific traits among different locust species.

Phase polyphenism has convergently evolved multiple times in acridid grasshoppers and is known to be expressed in species from several different subfamilies. In light of these independent evolutionary events, there are differences among taxa in phase characteristics that may prove informative in understanding the phase polyphenism-swarming correlation. Comparative studies both within and between locust species hold considerable promise for elucidating the respective roles of genetic factors, such as the expression of phase changes versus environmental factors, *e.g.*, weather, in promoting locust swarm formation. Different strains or populations of locusts of the same species are known to vary in their expression of phase polyphenism (reviewed in Pener & Yerushalmi 1998, see also Yerushalmi *et al.* 2001). These differences highlight the fact that while the expression of phase traits is environmentally determined, the underlying mechanisms and degree of plasticity are under genetic control and can differentially evolve via natural selection (or genetic drift) in different populations or species (Schlichting & Pigliucci 1998).

Laboratory-based comparisons of the expression of phase polyphenism between swarming and nonswarming species, or strains within a species, provide correlative support for an association between phase change and swarming. Chapuis *et al.* (unpub. data) found that the degree of expression of behavioral phase change in *L. migratoria* nymphs from a historically nonswarming population in France, was reduced relative to that of nymphs from a genetically distinct swarming Madagascan population. Similarly, Sword (2003) compared the expression of nymphal behavioral phase change between populations of the nonswarming *S. americana*, as well as between *S. americana* and the swarming *S. gregaria*. *S. americana* exhibited both developmental and geographic variation in its expression of behavioral phase change, but overall the degree of its expression was much reduced relative to that observed in *S. gregaria*. In both these cases, the patterns are consistent: swarming locusts express behavioral phase polyphenism to a greater extent than related, nonswarming taxa. Although informative, these studies unfortunately fail to provide any direct evidence of a causal role for phase polyphenism in locust swarm formation.

A series of intra- and interspecific comparative studies in *Schistocerca* provide the only direct evidence to date suggesting that density-dependent phase changes can play a causal role in locust swarm formation. In field and laboratory experiments, Sword (1999) showed that juveniles of the grasshopper, *Schistocerca lineata* (taxonomy according to Song 2004a; previously termed *Schistocerca emarginata*), express density-dependent warning coloration; they are commonly green when reared at low population density, but become an aposematic yellow-and-black, when reared at high density. Grasshoppers in these populations derive gut-content mediated toxicity to vertebrate predators simply by consuming their primary host plant, *Ptelea trifoliata* (Rutaceae) (Sword & Dopman 1999, Sword 2001). By contrast, *S. lineata* juveniles from genetically distinct populations associated with a different host plant, *Rubus trivialis* (Rosaceae), do not derive host-plant mediated deterrence to vertebrate predators (Dopman *et al.* 2002, Sword 2002). Juveniles in these palatable *Rubus*-feeding populations should not benefit from the expression of warning coloration at high population density, and as expected, the degree to which they change color with crowding is much less extensive than that of insects from unpalatable *Ptelea*-feeding populations (Sword 2002). Importantly, the cues mediating color change are independent of host-plant chemistry (Sword 2002) and differences in the ability to change color reflect genetic differences between the populations (Dopman *et al.* 2002). Thus, the ability to express density-dependent changes in juvenile coloration has differentially evolved between palatable and unpalatable *S. lineata* populations. In this case, the expression patterns of a specific density-dependent trait, namely color change, have been causally linked to local patterns of host plant use and predator defense.

The realization that the evolution of density-dependent traits can be directly related to local ecological factors prompted a follow-up comparative study in the desert locust, which also shows development of bright nymphal coloration upon crowding. Density-dependent color change in desert locust nymphs was similarly shown to function as density-dependent warning coloration, with deterrence to predators conferred by feeding on naturally occurring plants in pre-outbreak recession areas (Sword *et al.* 2000). As previously described, understanding the adaptive significance of this trait in the desert locust facilitated a broader understanding of the function of a number of phase traits acting in concert as an antipredator strategy. It also implies that some phase changes can interact with local ecological factors, such as plant community composition and resource distribution patterns, to promote locust swarm formation (Bouaïchi *et al* 1996, Collett *et al.* 1998, Babah & Sword 2004).

An important question arises about the generality of the relationship between locust phase traits and their role in swarm formation. Does phase polyphenism interact with local ecology to promote swarm formation in other locust species? This question can, at least in principle, be addressed using phylogenetic analyses. To this end, different researchers, utilizing different techniques, have been investigating the evolutionary origins of *Schistocerca*. A stated goal of these projects is to provide a phylogenetic framework for testing hypotheses about the relationship between phase polyphenism and locust swarm formation in *Schistocerca*.

The desert locust is the only species of *Schistocerca* found in the eastern hemisphere. All other species in the genus, of which there are likely more than 40 (Song 2004b), are found in the western hemisphere. This biogeographic scenario sharply contrasts with the distribution of genera within the subfamily to which *Schistocerca* belongs, the Cyrtacanthacridinae. With the exception of *Schistocerca* and the brachypterous Galapagos endemic genus, *Halmenus*, all of the other genera in the subfamily are found only in the eastern hemisphere. The major acridid subfamilies are thought to have diversified well after the Gondwanaland split, and most have either exclusively eastern or western-hemisphere distributions (Otte 1981). As such, a vicariance event is an unlikely explanation for this disjunctive distribution of genera within the subfamily. So how did such an enigmatic biogeographic distribution arise?

Based on morphological similarity between the Old World *S. gregaria* and members of the *S. americana* species complex in the New World, some authors suggested that *Schistocerca* originally diversified in the New World. This was followed by a west to east dispersal across the Atlantic Ocean by an ancestor of *S. gregaria*,

which gave rise to the single Old World species (Dirsh 1971). This scenario has become known as the "New World Origin" hypothesis (Song 2004b). Conversely, other authors suggested that *Schistocerca* most likely originated in the Old World, along with nearly all the other genera in its subfamily. The New World was then colonized one or more times by an ancestral form of *S. gregaria*, flying from east to west across the Atlantic and gave rise to an adaptive radiation of *Schistocerca* species in North and South America (Ritchie & Pedgley 1989, Kevan 1989, Vickery 1989). This scenario is referred to as the "Old World Origin" hypothesis.

The Old and New World Origin hypotheses make distinctly different predictions about the phylogenetic position of *S. gregaria*, relative to the other *Schistocerca* species (see Song 2004b, article this issue). Single or multiple west-to-east crossings of the Atlantic (New World Origin) predict that *S. gregaria* should be embedded within the New World clade and most closely related to the lineage that crossed the Atlantic. Multiple east to west crossings (Old World Origin) would also result in *S. gregaria* being embedded in the New World clade and sister to the New World lineage that arose from the most recent successful colonization event. By contrast, only a single east to west colonization event (Old World Origin) predicts that *S. gregaria* should be basal to the rest of the genus and sister to a monophyletic New World clade.

Song (2004b) conducted the first morphological cladistic analysis of *Schistocerca* and found *S. gregaria* to be nested within the New World clade of *Schistocerca* species that includes *S. americana* and the swarming New World species, *S. piceifrons* and *S. cancellata*. His analysis also indicated that the monophyletic *Schistocerca* clade was sister to the other New World representative of the Cyrtacanthacridinae, *Halmenus*. The morphological evidence suggests an intriguing biogeographic pattern within the subfamily, with *Schistocerca* and *Halmenus* being most closely related to cyrtacanthacridine genera in Australia and Asia. Based on this and other evidence, Song (2004b) proposed that the subfamily Cyrtacanthacridinae evolved in the Tertiary in Africa, with an eastward dispersal leading to the colonization of Asia, the Indo-Pacific, and Australian regions. This was followed by a trans-Pacific colonization event, from the Australasian regions to the Americas, where the genus *Schistocerca* subsequently diversified. Africa was then colonized via a trans-Atlantic crossing by an ancestral form of the extant *S. gregaria*. Thus, the morphological data support the New World Origin hypothesis.

Another group has been examining the molecular phylogenetics of *Schistocerca* using mitochondrial DNA sequence data. In contrast to the morphological data, the molecular data suggest a very different biogeographic scenario for the evolutionary origin of *Schistocerca*. The mtDNA data strongly support the Old World Origin hypothesis, with *S. gregaria* being basal and sister to all of the other members of the genus in the New World. Strikingly, the data also indicate that *Halmenus* is an endemic island form of *Schistocerca* (Lovejoy *et al.* 2006). *Halmenus* was found to be nested within the New World *Schistocerca* clade and sister to the other endemic *Schistocerca* species found on the Galapagos islands, *S. literosa* and *S. melanocera*. Thus, the molecular evidence implies that all of the New World *Schistocerca* species, as well as the presence of the genus, *Halmenus*, can be explained by a single trans-Atlantic colonization from Africa to the New World by an ancestral form of *S. gregaria*.

There are a number of avenues available to resolve this discrepancy between the morphological and molecular datasets. An obvious first step is to conduct a combined analysis of the 2 data sets. Combining independent data sets can help minimize error due to homoplasy within either of the individual data sets (Farris 1983, Miller *et al.* 1997), and may synergistically enhance the inference of phylogenetic relationships (Wahlberg *et al.* 2005). Another option is to conduct a separate phylogenetic analysis as an independent test, using nuclear DNA markers. Alternatively, the current morphological and molecular datasets make very different predictions about the degree of relatedness between *S. gregaria* and the New World taxa, particularly species in the americana complex (Harvey 1981). These hypotheses of genetic relatedness could be tested, for example, with genome-wide multilocus marker techniques such as AFLP (Vos *et al*. 1995, Mueller & Wolfenbarger 1999). Another possibility could be to assess divergence among taxa in traits such as the response to specific pheromones. For example, *a priori* hypotheses of how specific taxa should respond, based on their degree of genetic relatedness as predicted by the different phylogenies, could be tested in laboratory assays.

One intriguing possibility might be that the phylogenies inferred from both the molecular and morphological data do, in fact, reflect the true evolutionary history of the respective sampled traits. Consider the possibility of multiple Africa to New World trans-Atlantic colonisations, a seemingly likely scenario in light of the fact that desert locust swarms were observed to cross the Atlantic as recently as 1988 (Kevan 1989, Ritchie & Pedgley 1989, Rosenberg & Burt 1999). The current mtDNA phylogeny supports the notion of an east to west colonization event, but suggests that just one such event occurred. Given that mtDNA is maternally inherited, if multiple east to west crossings took place, but hybridizations following the initial colonization event occurred only between male desert locusts and female endemic *Schistocerca*, the mtDNA phylogeny would fail to reflect these subsequent hybridizations. On the other hand, a majority of the morphological traits examined by Song (2004b) are likely to be polygenic and determined by nuclear loci. Genes for these traits could feasibly spread following desert locust endemic hybridizations, and would yield a phylogenetic tree with *S. gregaria* as the sister to the taxon with whom a unidirectional introgression most recently occurred. It should be noted that laboratory crossings between *S. gregaria* and other members of the New World americana complex either failed to result in viable progeny or resulted only in females thought to have arisen parthogenetically in the absence of fertilization (Jago *et al.* 1979). However, the apparent lack of introgression among extant taxa does not necessarily rule out its possibility among ancestral forms.

A robust *Schistocerca* phylogeny will provide an important tool for future comparative studies of phase polyphenism. It will also be used to better understand the mechanisms involved in locust swarm formation. Not all *Schistocerca* species swarm, but we do not yet know why. Some nonswarming species may retain genetic variation for the expression of density-dependent traits that play a causal role in swarm formation, but simply inhabit environments that are not conducive to swarm formation. Alternatively, nonswarming species may have lost the genetic capacity to become swarming locusts, despite occurring in environments that are conducive to swarm formation (Sword 2003, Song 2004b, Lovejoy *et al.* submitted). Resolving the current phylogenetic conflict will enable the use of *Schistocerca* as a model system to study the relationship between genetic and environmental factors in locust swarm formation.

Development of common resources

The field of phase research has benefited considerably from cooperation between laboratories and the sharing of techniques, expertise and facilities. It is vital that such cooperation continues,

but consideration should also be given to pooling resources for the development of major new initiatives. Foremost among such initiatives is the development of an expressed sequence tag (EST) database of the central nervous system of *S. gregaria* for use in the molecular analysis of phase change. ESTs are short DNA sequences of larger protein-coding genes that can be useful tools in genome mapping and gene discovery.

The molecular bases of phase change are yet to be elucidated. Levels of a few peptide precursor gene transcripts have been shown to differ between solitarious and gregarious desert locusts (Rahman *et al.* 2003; Simonet *et al.* 2004, 2005; Claeys *et al.* 2005), and recent work on *L. migratoria* has shown > 500 gene expression differences between gregarious and solitarious insects, most of which have no homologue in already sequenced insect genomes (Kang *et al.* 2004). Whereas a more targeted approach to investigating phase-dependent regulation of gene expression, founded on knowledge of the detailed time course of phase change and known neural and hormonal pathways (see above), is likely to clarify this situation, the fact that the locust genome is not yet sequenced and is huge (several times larger than the human genome) provides a major impediment. To help overcome this, Jozef Vanden Broeck, Arnold De Loof and colleagues at Leuven University in Belgium, with copromotion by Steve Simpson (Oxford, now Sydney), Malcolm Burrows (Cambridge) and Wolfgang Blenau (Potsdam), have initiated a project to generate an EST library, representing a large number of transcripts expressed in the desert locust central nervous system. After generating a large number of partial cDNA (5′ expressed sequence tags), a database will be built which will provide the basis for bioinformatic analyses, as well as the development of microarrays for studying differential gene expression within the nervous system of locusts as they change phase.

The EST library project recently received Belgian funding. This will guarantee the initiation of the project, but further funds are required for its completion. The hope is that interested groups around the world will use the Belgians' success as the basis for requesting contributing funds from their own national funding agencies. The database will be held and managed by the group at Leuven and once completed, will provide a core international resource.

Another limiting resource for phase research is solitarious locust cultures. Presently, major solitarious cultures are found in the UK (Oxford), Australia (Sydney), Belgium (Leuven), Israel (Tel-Aviv) and Kenya (ICIPE). The rearing methods used differ between groups, as do the origins of the locusts. As discussed above in the context of semiochemistry, these differences need to be considered when comparing results from different laboratories, and where possible key findings should be verified using more than one culture. Maintenance of solitarious locust cultures is laborious and expensive. Consortia of labs need to consider ways in which to secure the future of these cultures. For example, national and international funding agencies are likely to look more favorably upon applications indicating that solitarious cultures will be used to support multiple projects within the region.

Future research priorities

1) Determine the neural and hormonal bases of behavioral phase change. Having a quantitative understanding of the time course of behavioral phase change and also of its triggering mechanisms, provides the framework for exploring underlying neurohormonal (and genetic) mechanisms (Simpson *et al.* 1999). Identifying the hind leg as a site of input for behavioral gregarization (Simpson *et al.* 2001) was a significant breakthrough, but there is still much to be done. Exploring the potential role of serotonin in the initial stages of behavioral phase change and how this may elicit functional changes in the central nervous system (Rogers *et al.* 2004) is a major priority for research. Understanding how the modifications of neural circuits within the central nervous system, that arise as a consequence of phase change, produce such striking differences in behavior is also important (*e.g.*, Fuchs *et al.* 2003, Matheson *et al.* 2004).

2) A major aim has been to understand patterns of gene expression that accompany and control phase change. Research into behavioral phase change and its controlling mechanisms have set the scene for such an analysis, and the EST database will greatly facilitate the project. Synergistic benefits will arise from advances in understanding of the neurohormonal control of phase change.

3) A related priority is to explore the developmental genetics of phase change. What is the maternally produced gregarizing agent (McCaffery *et al.* 1998) and how does it affect embryonic development such that larvae emerge into the gregarious phase?

4) Determine the ecological correlates of phase change across spatial scales. We know that small-scale features of the habitat, such as resource abundance, quality and distribution, are critical in determining phase transition within local populations (Collett *et al.* 1998; Despland *et al.* 2000; Despland & Simpson 2000a,b; Despland *et al.* 2004; Babah & Sword 2004), but how does this relate to larger spatial scales, and how can such knowledge be used to inform prediction and management of locust outbreaks?

5) How are populations of locusts structured genetically with respect to the capacity to change phase? Initial work on desert locust has been undertaken using nuclear markers (Ibrahim *et al.* 1996, 2000; Ibrahim 2001), while Chapuis *et al.* (unpub.) have used microsatellites to show genetic differences in populations of *L. migratoria* that differ in their ability to gregarize.

6) Finally, as evidenced by the focus on *S. gregaria* in this paper, detailed studies of the expression of phase polyphenism in other species are needed. Even within a single individual, different stimuli and physiological mechanisms can mediate the expression of different phase traits. How general are the underlying stimuli, mechanisms, and ecological functions of different phase traits among phylogenetically disparate locust species? Do phase changes in some traits play a causal role in swarm formation? How do different phase characteristics map onto phylogenies within the genus *Schistocerca* and within the Acrididae at large? These are all key questions for future research.

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Literature Cited

Applebaum S.W., Heifetz Y. 1999. Density-dependent physiological phase in insects. Annual Review of Entomology 44: 317-341.

Applebaum S.W., Avisar E., Heifetz Y. 1997. Juvenile hormone and locust phase. Archives of Insect Biochemistry and Physiology 35: 375-391.

Ayali A., Pener M.P., Girardie J. 1996a. A comparative study of neuropeptides from the corpora cardiaca of solitary and gregarious *Locusta*. Archives of Insect Biochemistry and Physiology 31: 439-450.

Ayali A., Pener M.P., Sowa S.M., Keeley L.L. 1996b. Adipokinetic hormone content of the corpora cardiaca in gregarious and solitary migratory locusts. Physiological Entomology 21: 167-172.

Babah M.A.O., Sword G.A. 2004. Linking locust gregarization to local resource distribution patterns across a large spatial scale. Environmental Entomology 33: 1577-1583.

Bailey N.W., Gwynne D.T., Ritchie M.G. 2005 Are solitary and gregarious mormon crickets (*Anabrus simplex*, Orthoptera, Tettigoniidae) genetically distinct? Heredity 95: 166-173.

Bouaïchi A., Roessingh P., Simpson S.J. 1995. An analysis of the behavioural effects of crowding and re-isolation on solitary-reared adult desert locusts (*Schistocerca gregaria*) and their offspring. Physiological Entomology 20: 199-208.

Bouaïchi A., Simpson S.J., Roessingh P. 1996. The influence of environmental microstructure on the behavioural phase state and distribution of the desert locust *Schistocerca gregaria*. Physiological Entomology 21: 247-256.

Chapuis, M-P., Auge-Sabatier A., Estoup A., Foucart A., Lecoq M., Michalakis Y. 2005. Genetic determinism of gregarization in the migratory locust *Locusta migratoria*. Abstract, Metaleptea Special Meeting Issue. 9th International Conference Orthopterists' Society. Canmore, Alberta, Canada.

Claeys I., Simonet G., Breugelmans B., Van Soest S., Franssens V., Sas F., De Loof A., Vanden Broeck J. 2005. Quantitative real-time RT-PCR analysis in desert locusts reveals phase dependent difference in neuroparsin transcript levels. Insect Molecular Biology 14: 415-422.

Clark L.R. 1949. Behaviour of swarm hoppers of the Australian plague locust (*Chortoicetes terminifera* Walk.). CSIRO Bulletin 245: 1-27.

Clynen E., Baggerman G., Veelaert D., Cerstiaens A., Van der Horst D., Harthoorn L., Derua R., Waelkens E., De Loof A., Schoofs L. 2001. Peptidomics of the pars intercerebralis-corpus cardiacum complex of the migratory locust, *Locusta migratoria*. European Journal of Biochemistry 268: 1929-1939.

Collett M., Despland E., Simpson S.J., Krakauer D.C. 1998. Spatial scales of desert locust gregarization. Proceedings of the National Academy of Sciences of the United States of America 95: 13052-13055.

Deng A.L., Torto B., Hassanali A., Ali E.E. 1996. Effects of shifting to crowded or solitary conditions on pheromone release and morphometrics of the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). Journal of Insect Physiology 42: 771-776.

Despland E., Simpson S.J. 2000a. Small-scale vegetation patterns in the parental environment influence the phase state of hatchlings of the desert locust. Physiological Entomology 25: 74-81.

Despland E, Simpson SJ. 2000b. The role of food distribution and nutritional quality in behavioural phase change in the desert locust. Animal Behaviour 59: 643-652.

Despland E., Simpson S.J. 2005a. Surviving the change to warning colouration: density-dependent polyphenism suggests a route for the evolution of aposematism. Chemoecology 15: 69-75.

Despland E., Simpson S.J. 2005b. Food choices of solitarious and gregarious locusts reflect cryptic and aposematic antipredator strategies. Animal Behaviour 69: 471-479.

Despland E., Rosenberg J., Simpson S.J. 2004. Landscape structure and locust swarming: a satellite's view. Ecography 27: 381-391.

Despland E., Collett M., Simpson S.J. 2000. Small-scale processes in Desert Locust swarm formation: how vegetation patterns influence gregarization. Oikos 88: 652-662.

Dirsh V.M. 1974. Genus *Schistocerca* (Acridomorpha, Insecta). W. Junk, The Hague.

Dopman E.B., Sword G.A., Hillis D.M. 2002. The importance of the ontogenetic niche in resource-associated divergence: Evidence from a generalist grasshopper. Evolution 56: 731-740.

Dorn A., Ress C., Sickold S., Wedekind-Hirschberger S. 2000. Arthropoda – Insecta: endocrine control of phase polymorphism, pp. 205-253. In: Dorn A. (Ed.) Progress in Developmental Endocrinology. John Wiley, Chichester.

Farris J.S. 1983. The logical basis of phylogenetic analysis, pp. 7-36. In: Platnick N.I., Funk V.A. (Eds), Advances in cladistics. Columbia University Press, New York.

Fatouros N.E., Huigens M.E., van Loon J.J.A., Dicke M., Hilker M. 2005. Butterfly anti-aphrodisiac lures parasitic wasps. Nature 433: 704.

Ferenz H-J., Seidelmann K. 2003. Pheromones in relation to aggregation and reproduction in desert locusts. Physiological Entomology 28: 11-18.

Fuchs E., Kutsch W., Ayali A. 2003. Neural correlates to flight-related density–dependent phase characteristics in locusts. Journal of Neurobiology 57: 152-162.

Gamberale G., Tullberg B.S. 1998. Aposematism and gregariousness: the combined effect of group size and coloration on signal repellence. Proceedings Royal Society of London Series B, Biological Sciences 265: 889-894.

Gwynne D.T. 2001. Katydids And Bush-crickets: Reproductive Behavior and Evolution of the Tettigoniidae. Cornell University Press, Ithaca.

Hägele B.F., Simpson S.J. 2000. The influence of mechanical, visual and contact chemical stimulation on the behavioural phase state of solitary desert locusts (*Schistocerca gregaria*). Journal of Insect Physiology 46: 1295-1301.

Hägele B.F, Oag V., Bouaïchi A., McCaffery A.R., Simpson S.J. 2000. The role of the female accessory glands in maternal inheritance of phase in the desert locust, *Schistocerca gregaria*. Journal of Insect Physiology 46: 275-280.

Hägele B.F., Wang F-H., Sehnal F., Simpson S.J. 2004. Effects of crowding, isolation, and transfer from isolation to crowding on the total ecdysteroid content of eggs in *Schistocerca gregaria*. Journal of Insect Physiology 50: 621-628.

Hartfelder K., Emlen D.J. 2005. Endocrine control of insect polyphenism, pp. 651-703. In: Gilbert L.I., Iatrou K., Gill S.S. (Eds) Comprehensive Molecular Insect Science, Vol. 3, Endocrinology. Elsevier Pergamon, Oxford.

Harvey A.W. 1981. A reclassification of the *Schistocerca americana* complex (Orthoptera: Acrididae). Acrida 10: 61-77.

Hassanali A., Njagi P.G.N., Bashir M.O. 2005. Chemical ecology of locusts and related acridids. Annual Review of Entomology 50: 223-245.

Hoste B., Simpson S.J., Tanaka S., Zhu D-H., De Loof A., Breuer M. 2002. Effects of [His7]-corazonin on the phase state of isolated-reared (solitarious) *Schistocerca gregaria*. Journal of Insect Physiology 48: 981-990.

Hunter D.M. 2004 Advances in the control of locusts (Orthoptera: Acrididae) in eastern Australia: from crop protection to preventative control. Australian Journal of Entomology 43: 293-303.

Ibrahim K.M. 2001. Plague dynamics and population genetics of the desert locust: can turnover during recession maintain population genetic structure? Molecular Ecology 10: 581-591.

Ibrahim K.M., Nichols R.A., Hewitt G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77: 282-291.

Ibrahim K.M., Sourrouille P., Hewitt G.M. 2000. Are recession populations of the desert locust (*Schistocerca gregaria*) remnants of past swarms? Molecular Ecology 9: 783-791.

Ignell R., Couillaud F., Anton S. 2001. Juvenile-hormone-mediated plasticity of aggregation behaviour and olfactory processing in adult desert locusts. Journal of Experimental Biology 204: 249-259.

Islam M.S., Roessingh P., Simpson S.J., McCaffery A.R. 1994a. Effects of population density experienced by parents during mating and oviposition on the phase of hatchling desert locusts, *Schistocerca gregaria*. Proceedings Royal Society of London Series B, Biological Sciences 257: 93-98.

Islam M.S., Roessingh P., Simpson S.J., McCaffery A.R. 1994b. Parental effects on the behaviour and colouration of nymphs of the desert locust *Schistocerca gregaria*. Journal of Insect Physiology 40: 173-181.

Jago N.D, Antoniou A., Scott P. 1979. Laboratory evidence showing the separate species status of *Schistocerca gregaria*, *americana* and *cancellata* (Acrididae, Crytacanthacridinae). Systematic Entomology 4: 133-142.

Kang L., Chen X.Y., Zhou Y., Liu B.W., Zheng W., Li R.Q., Wang J., Yu J. 2004. The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. Proceedings National Academy of Sciences of the United States of America 101: 17611-17615.

Kevan D.K.McE. 1989. Transatlantic travellers. Antenna 13: 12-15.

Key K.H.L. 1950. A critique on the phase theory of locusts. Quarterly Review of Biology 25: 363-407.

Krause J., Ruxton G.D. 2002. Living In Groups. Oxford University Press, Oxford.

Lester R.L., Grach C., Pener M.P., Simpson S.J. 2005. Stimuli inducing gregarious colouration and behaviour in nymphs of *Schistocerca gregaria*. Journal of Insect Physiology 51: 737-747.

Lorch P.D., Sword G.A., Gwynne D.T., Anderson G.T. 2005. Radiotelemetry reveals differences in individual movement patterns between outbreak and non-outbreak Mormon cricket populations. Ecological Entomology, 30: 548-555.

Lovejoy N., Mullen S.P., Sword G.A., Chapman R.F., Harrison R. 2006. Ancient trans-Atlantic flight explains locust biogeography: molecular phylogenetics of the locust genus *Schistocerca*. Proceedings Royal Society London Series B, Biological Sciences 273: 767-774.

Luber K., Wieting J., Zeeck E., Ferenz, H-J. 1993. Isolation and characterization of a volatile aromatic infochemical released by sexual maturing, gregarious male desert locusts, *Schistocerca gregaria*. Verhandlungen der Deutschen Zoologischen Gesellschaft 86: 261.

Matheson T., Rogers S.M., Krapp H.G. 2004. Plasticity in the visual system is correlated with a change in lifestyle of solitarious and gregarious locusts. Journal of Neurophysiology 91: 1-12.

McCaffery A.R., Simpson S.J., Islam M.S., Roessingh P. 1998. A gregarizing factor present in egg pod foam of the desert locust *Schistocerca gregaria*. Journal of Experimental Biology 201: 347-363.

Miller J.S., Brower A.V.Z., DeSalle R. 1997. Phylogeny of the neotropical moth tribe Josiini (Notodontidae: Dioptinae): comparing and combining evidence from DNA sequences and morphology. Biological Journal of the Linnean Society 60: 297-316.

Mueller U.G., Wolfenbarger L.L. 1999. AFLP genotyping and fingerprinting. Trends in Ecology and Evolution 14: 389-394.

Njagi P.G.N., Torto B. 2002. Evidence for a compound in Comstock-Kellog glands modulating premating behavior in male desert locust, *Schistocerca gregaria*. Journal of Chemical Ecology 28: 1065-1074.

Njagi P.G.N., Torto B., Obeng-Ofori D., Hassanali A. 1996. Phase-independent responses to phase-specific aggregation pheromone in adult desert locusts, *Schistocerca gregaria* (Orthoptera: Acrididae). Physiological Entomology 21: 131-137.

Obeng-Ofori D., Torto B., Hassanali A. 1993. Evidence for mediation of two releaser pheromones in aggregation behavior of the gregarious desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Journal of Chemical Ecology 19: 1665-1676.

Otte D. 1981. The North American Grasshoppers, volume 1 - Acrididae: Gomphocerinae and Acridinae. Harvard University Press, Cambridge.

Pener M.P. 1991. Locust phase polymorphism and its endocrine relations. Advances in Insect Physiology 23: 1-79.

Pener M.P., Yerushalmi Y. 1998. The physiology of locust phase polymorphism: an update. Journal of Insect Physiology 44: 365-377.

Pener M.P., Ayali A., Ben-Ami E. 1992. Juvenile hormone is not a major factor in locust phase changes, pp. 125-134. In: Mauchamp B, Couillaud F, Baehr J.C. (Eds) Insect Juvenile Hormone Research. Institut National de la Recherché Agronomique, Paris.

Rahman M.M., Vanden Bosch L., Baggerman G., Clynen E., Hens K., Hoste B., Meylaers K., Vercammen T., Schoofs L., De Loof A., Breuer M. 2002. Search for peptidergic molecular markers in hemolymph of crowd- (gregarious) and isolated-reared (solitary) desert locusts, *Schistocerca gregaria*. Peptides 23: 1907-1914.

Rahman M.M., Vandingenen A., Begum M., Breuer M., De Loof A., Huybrechts R. 2003. Search for phase specific genes in brain of desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae) by differential display polymerase chain reaction. Comparative Biochemistry and Physiology A: Molecular Integrative Physiology 135: 221-228.

Ritchie M., Pedgley D. 1989. Desert locusts cross the Atlantic. Antenna, 13: 10-12.

Roessingh P., Bouaichi A., Simpson S.J. 1998. Effects of sensory stimuli on the behavioural phase state of the desert locust, *Schistocerca gregaria*. Journal of Insect Physiology 44: 883-893.

Rogers S.M., Matheson T., Despland E., Dodgson T., Burrows M., Simpson S.J. 2003. Mechanosensory-induced behavioural gregarization in the desert locust *Schistocerca gregaria*. Journal of Experimental Biology 206: 3991-4002.

Rogers S.M., Matheson T., Sasaki K., Kendrick K., Simpson S.J., Burrows M. 2004. Substantial changes in central nervous system neurotransmitters and neuromodulators accompany phase change in the locust. Journal of Experimental Biology 207: 3603-3617.

Rosenberg J., Burt P.J.A. 1999. Windborne displacements of Desert Locusts from Africa to the Caribbean and South America. Aerobiologia 15: 167-175.

Schlichting C.D., Pigliucci M. 1998. Phenotypic Evolution: a Reaction Norm Perspective. Sinauer Associates, Sunderland.

Schmidt G.H., Krempien W., Johannes B. 1987. Studies on the secretion of the prothoracic epidermal gland in *Acrotylus patruelis* (Insecta: Saltatoria: Acrididae). Zoologischer Anzeiger 219: 357-368.

Seidelmann K., Ferenz H-J. 2002. Courtship-inhibition pheromone in desert locusts, *Schistocerca gregaria*. Journal of Insect Physiology 48: 991-996.

Seidelmann K., Luber K., Ferenz H-J. 2000. Analysis of release and role of benzyl cyanide in male desert locusts, *Schistocerca gregaria*. Journal of Chemical Ecology 26: 1897-1910.

Seidelmann K., Warnstorff K., Ferenz H-J. 2005. Phenylacetonitrile is a male specific repellent in gregarious desert locusts, *Schistocerca gregaria*. Chemoecology 15: 37-43.

Seidelmann K., Weinert H., Ferenz H-J. 2003. Wings and legs are production sites for the desert locust courtship inhibition pheromone, phenylacetonitrile. Journal of Insect Physiology 49: 1125-1133.

Simonet G., Claeys I., Breugelmans B., Van Soest S., De Loof A., Vanden Broeck J. 2004. Transcript profiling of pacifastin-like peptide precursors in crowd- and isolated-reared desert locusts. Biochemical and Biophysical Research Communications 317: 565-569.

Simonet G., Breugelmans B., Proost P., Claeys I., Van Damme J., De Loof A., Vanden Broeck J. 2005. Characterization of two novel pacifastin-like peptide precursor isoforms in the desert locust (*Schistocerca gregaria*): cDNA cloning, functional analysis and real-time RT-PCR gene expression studies. Biochemical Journal 388: 281-289.

Simpson S.J., Despland E., Hägele B.F., Dodgson T. 2001. Gregarious behavior in desert locusts is evoked by touching their back legs. Proceedings National Academy of Sciences of the United States of America 98: 3895-3897.

Simpson S.J., McCaffery A.R., Hägele B.F. 1999. A behavioural analysis of phase change in the desert locust. Biological Reviews 74: 461-480.

Simpson S.J., Raubenheimer D., Behmer S.T., Whitworth A., Wright G.A. 2002. A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust, *Schistocerca gregaria*. Journal of Experimental Biology 205: 121-129.

Song H. 2004a. Revision of the Alutacea Group of genus *Schistocerca* (Orthoptera: Acrididae: Cyrtacanthacridinae). Annals of the Entomological Society of America 97: 420-436.

Song H. 2004b. On the origin of the desert locust *Schistocerca gregaria* (Forskål) (Orthoptera : Acrididae : Cyrtacanthacridinae). Proceedings Royal Society of London, Series B, Biological Sciences 271: 1641-1648.

Sword G.A. 1999. Density-dependent warning coloration. Nature 397: 217.

Sword G.A. 2001.Tasty on the outside, but toxic in the middle: grasshopper regurgitation and host plant-mediated toxicity to a vertebrate predator. Oecologia 120: 416-421.

Sword G.A. 2002. A role for phenotypic plasticity in the evolution of aposematism. Proceedings Royal Society of London Series B, Biological Sciences 269: 1639-1644.

Sword G.A. 2003. To be or not to be a locust? A comparative analysis of behavioral phase change in nymphs of *Schistocerca americana* and *S. gregaria*. Journal of Insect Physiology 74: 709-717.

Sword G.A. 2005. Local population density and the activation of movement in migratory band-forming Mormon crickets. Animal Behaviour 69: 437-444.

Sword G.A., Dopman E.B. 1999. Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata* (=*lineata*) (Orthoptera: Acrididae). Oecologia 120: 437-445.

Sword G.A., Simpson S.J. 2000. Is there an intraspecific role for density-dependent colour change in the desert locust? Animal Behaviour 59: 861-870.

Sword G.A., Lorch P.D., Gwynne D.T. 2005. Migratory bands give crickets protection. Nature 433: 703.

Sword G.A., Simpson S.J., El Hadi O.T.M., Wilps H. 2000. Density-dependent aposematism in the desert locust. Proceedings Royal Society of London. Series B, Biological Sciences 267: 63-68.

Tanaka S. 2000. The role of [His^7]-corazonin in the control of body-colour polymorphism in the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae). Journal of Insect Physiology 46: 1169-1176.

Tanaka S. 2001. Endocrine mechanisms controlling body-color polymorphism in locusts. Archives of Insect Biochemistry and Physiology 47: 139-149.

Tanaka S., Zhu D-H., Hoste B., Breuer M. 2002. The dark-color inducing neuropeptide [His^7]-corazonin, causes a shift in morphometric characteristics towards the gregarious phase in isolated-reared (solitarious) *Locusta migratoria*. Journal of Insect Physiology 48: 1065-1074.

Tawfik A.I., Tanaka S., De Loof A., Schoofs L., Baggerman G., Waelkens E., Derua R., Milner Y., Yerushalmi Y., Pener M.P. 1999. Identification of the gregarization-associated dark-pigmentotropin in locusts through an albino mutant. Proceedings National Academy of Sciences of United States of America 96: 7083-7087.

Torto B., Obeng-Ofori D., Njagi P.G.N., Hassanali A., Amiani H. 1994. Aggregation pheromone system of adult gregarious desert locust *Schistocerca gregaria* (Forskal). Journal of Chemical Ecology 20: 1749-1762.

Uvarov B. 1966. Grasshoppers and Locusts Vol. 1. Cambridge University Press, Cambridge.

Vickery V.R. 1989. The biogeography of Canadian Grylloptera and Orthoptera. Canadian Entomologist 121: 389-424.

Vos P., Hogers R., Bleeker M., Reijans M., Van Der Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Wahlberg N., Braby M.F., Brower A.V.Z., de Jong R., Lee M., Nylin S., Pierce N., Sperling F.A.H., Vila R., Warren A.D., Zakharov E. 2005. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. Proceedings of the Royal Society Series B-Biological Sciences 272: 1577-1586.

Wedekind-Hirschberger S., Sickold S., Dorn A. 1999. Expression of phase-specific haemolymph polypeptides in a laboratory strain and field catches of *Schistocerca gregaria*. Journal of Insect Physiology 45: 1097-1103.

Whitman D.W. 1990. Grasshopper chemical communication, pp. 357-391. In: Chapman R.F., Joern A. (Eds) Biology of Grasshoppers. John Wiley & Sons, New York.

Wilson K. 2000. How the locust got its stripes: the evolution of density-dependent aposematism. Trends in Ecology and Evolution 15: 88-90.

Wilson K., Thomas M.B., Blanford S., Doggett M., Simpson S.J., Moore S.L. 2002. Coping with crowds: Density-dependent disease resistance in desert locusts. Proceedings National Academy of Sciences of United States of America 99: 5471-5475.

Yerushalmi Y., Tauber E., Pener M.P. 2001. Phase polymorphism in *Locusta migratoria*: the relative effects of geographical strain and albinism on morphometrics. Physiological Entomology 26: 95-105.