ORIGINAL RESEARCH - RESUBMISSION

Chemical Ecology and Olfaction in Short-Horned Grasshoppers (Orthoptera: Acrididae)

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in locust phase has therefore driven research into Acrididae chemical ecology. In addition, because insecticides used to control locusts have negative impacts on human health and the environment (Byers 1991; Zhang et al. 2019), the use of alternative control methods such as pheromone traps, genetically modified pests, and entomopathogenic fungi, that are more species-specific and environmentally safer are being investigated.

As chemical communication has a central role in triggering the switch between the two locust phases (Hassanali et al. 2005), the study of chemical ecology provides the basis for predicting when swarming is likely to happen and potentially controlling outbreaks. At sensilla, which are the sensory organs that project through the insect exoskeleton, sensory neurons and proteins respond to specific tastes or smells. Pioneering studies identified compounds involved in gregarization (i.e., aggregation pheromones) using gas-chromatograph and mass spectrometry (GC-MS), and explored the mechanisms of perception using physiological and behavioral observations (Mahamat et al. 1993; Hansson et al. 1996; Ochieng et al. 1998; Niassy et al. 1999; Ochieng and Hansson 1999). Physiological responses to specific chemical stimuli can be investigated using electrophysiological techniques including electroantennography (EAG) (Torto et al. 1994; Njagi et al. 1996; Chen et al. 2004) and single sensillum recordingluaering studies identified compounds in5nsillumi

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Chorthippus grasshopper males use CHCs to identify potential mates and have been observed touching the body and antennae of females with their antennae before copulation (Ritchie 1990; Finck et al. 2016b). Conversely, olfactometer studies show that detection of odour cues results in the attraction of many grasshopper species towards volatiles of conspecifics (*L. migratoria*: Guo et al. 2011; *S. gregaria*: Inayatullah et al. 1994; Ould Ely et al. 2006; *Schistocerca americana*: Stahr et al. 2013; *Dociostaurus maroccanus*: Guerrero et al. 2019) and host plants (*S. gregaria*: Njagi and Torto 1996; *Melanoplus sanguinipes*: Hopkins and Young 1990). The specific chemical compounds identified and their behavioral effect is described in the next section.

Chemical Signals Perceived and their Effect on Grasshopper Behavior

Cuticular Hydrocarbons (CHCs) Cuticular hydrocarbons are derived from the insect exoskeleton and have a primary function of preventing water loss (Blomquist et al. 2018). Cuticular hydrocarbons are relatively long carbon chains (21 to >40 carbons) with single (alkanes) or double bonds (alkenes and alkadienes) sometimes including methyl branches (Gibbs and Rajpurohit 2010; Blomquist et al. 2018). In *L. migratoria*, straight-chain 25–33 carbon alkanes are important water-proofing agents, and disruption of CHC synthesis results in high mortality due to severe water-loss (Yu et al. 2016). Cuticular hydrocarbons also provide a barrier against fungi and insecticides (Wu et al. 2020; Zhang et al. 2021).

Short-horned grasshoppers have species-specific qualitative and quantitative CHC profiles. The locusts *S. gregaria* and *L. migratoria migratodiodes* each have characteristic CHCs (Lockey and Oraha 1990) with the former species dominated by straight-chain alkanes whereas the latter dominated by mono- and dimethyl-alkanes (with some compounds being specific). In the sympatric grasshoppers

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2019; Guo et al. 2020) (Fig. 2). Although several compounds such as veratrole, guaiacol, benzaldehyde, hexanoic acid, nonanal, and (*Z*)-3-nonen-1-ol are found in the pheromonal blends of different locust species (e.g., *S. americana*, *S. gregaria*, *S. piceifrons*, *L. migratoria*), significant differences in emission dynamics are observed within and among them (Niassy et al. 1999; Mahamat et al. 2000; Stahr et al. 2013; Stahr and Seidelmann 2016; Wei et al. 2017). Relative concentrations in pheromone cocktails provide signals that are

(Tanaka et al. 2018; Sakamoto et al. 2019), and development (Ellis et al. 1965; Mahamat et al. 1993; Stahr et al. 2013). These group behaviors are thought to help secure resources (e.g., food, oviposition substrate) and enhance

or more (Ellis et al. 1965; Carlisle et al. 1969). When gibberellic acid is added to the senescent leaves, it accelerates sexual maturation in *S. gregaria* but interestingly, delayed development was observed when the compound was added to green leaves (Ellis et al. 1965). The delayed development is possibly related to the phytotoxicity of gibberellic acid above a certain threshold. For example in *L. migratoria*, the rate of consumption, nymphal development, and oviposition of newly emerged females reduced as the concentration of this compound increased (Abdellaoui et al. 2009, 2015). These studies show that gibberellic acid signals optimal time for development and reproduction, but it also can be toxic above certain limits.

Plant-derived chemicals can influence where grasshoppers oviposit their eggs. *Schistocerca gregaria* females presented with a choice of sand containing either leaf extracts of their host plants (orchard grass, cabbage, sorghum, romaine lettuce, Japanese mustard spinach, or silver grass), frass extract from other locusts (*S. gregaria*, *L. migratoria,* and *Patanga succincta*) or water (control), laid more eggs in the control sand than in the sand containing extracts (Tanaka et al. 2019). This preference for oviposition sites is related to egg-hatching rate and embryonic development (egg size and antennal length) that were significantly reduced by the presence of frass and plant extracts. A similar inhibition effect has been observed in *L. migratoria* (Sugahara et al. 2021), the choice of oviposition sites was not influenced by phase polyphenism or bacterial activity in either species (Tanaka et al. 2019; Sugahara et al. 2021). The compounds inducing oviposition inhibition are unknown but may involve toxic compounds such as alkaloids.

Chemical Biosynthesis in Grasshoppers In insects, CHCs are synthesised from fatty acids and terpenoid lipids, in specialized cells called oenocytes present in the abdomen or fat bodies (Blomquist et al. 2018). Synthesis of different types of CHCs involves a variety of catalysts including fatty acid synthase, reductases, and elongases (Blomquist et al. 2018). In short-horned grasshoppers, biosynthetic pathways of CHC formation have only been studied in *L. migratoria* (Yu et al. 2016; Wu et al. 2020) and *S. gregaria* (Diehl 1975). Two genes from the superfamily of cytochrome P450 enzymes are expressed specifically in oenocytes and they

receptors (Nowi ska and Bro ek 2017). Sensilla are attached with either a flexible or inflexible base (Fig. 3). Sensilla inferred as mechano-sensitive typically have a flexible socket for movement detection, whereas sensilla with inflexible sockets probably detect humidity, temperature, smells, or taste (Nowi ska and Bro ek 2017). Some sensilla such as

(Table 1). Here, we use terminology derived from the study of the locusts *S. gregaria* and *L. migratoria* (Altner et al. 1981; Ochieng et al. 1998; Jin et al. 2005; Zhou et al. 2008). As arable pests, the functions of their sensilla have received the most detailed investigation with physiological (Altner et al. 1981; Ochieng and Hansson 1999) and transcriptomic (Jiang et al. 2017; Jin et al. 2005; Zhou et al. 2008) approaches providing an evidential basis for functional inference. Trichoid sensilla (Fig. 3e) that are responsible for olfaction in locusts, are slender, hair-like sensilla with pores on their walls (Ochieng et al. 1998; Ochieng and Hansson 1999). Much longer than trichoid sensilla with a flexible socket but no pores (Table 1, Fig. 3a) are the hair sensilla responsible for mechanoreception (Bland 1989; Chen et al. 2003; Li et al. 2007; Yu et al. 2011; Zhou et al. 2008). Sensilla chaetica (Fig. 3b) associated with mechano- and gustatory-receptions are thick and peg-like with a flexible socket, ribbed wall, and an apical pore (Bland 1989; Blaney and Chapman 1969; Chen et al. 2003; Jin et al. 2006; Li et al. 2007; Ochieng et al. 1998; Yu et al. 2011; Zhou et al. 2008). Basiconic olfactory sensilla are wall-pored (Fig. 3c and d), but vary in shape among species (Bland 1989; Chapman 1989; Chen et al. 2003; Jin et al. 2006; Li et al. 2007; Ochieng et al. 1998). Sensilla coeloconica consists of a short peg in a cavity commonly considered to be temperature and humidity receptors (Nowiska and Broek 2017; Jiang et al. 2018). Two typeeloa coeloconicay r L. mi5(g).5(r)2or8(i6(a)TJ/T11 1 T

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a single apical pore (Table 1). These sensilla within pits are responsible for detecting smells and temperature, and humidity and temperature, respectively (Altner et al. 1981). The response of wall-pored coeloconica to olfactory stimuli has also been confirmed in *S. gregaria* (Ochieng and Hansson 1999). Wall-pored coeloconica seem to be as common on grasshopper antennae as aporous sensilla (Altner et al. 1981; Bland 1989; Chapman 1989; Chen et al. 2003; Greenwood and Chapman 1984; Li et al. 2007).

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sensilla; Zaim et al. 2013). In another study, desert species *B. argentatus*, *L. coquilletti,* and *C. parviceps* have fewer sensilla (800 – 2000) on their antennae than species living in more equitable habitats such as *Chorthippus curtipennis* and *Metaleptea brevicornis* (4000 – 8500 sensilla; Bland 1989). Species with a limited diet range or desert grasshoppers may be exposed to fewer chemical compounds than species that are polyphagous or living in more complex environments.

Male grasshoppers have more olfactory sensilla on their antennae than females in most grasshopper species studied (80%, n = 75; Chen et al. 2003; Bland 1989; Li et al. 2007). Higher electrophysiological responses to chemical signals in males have also been observed in some of the studies using single sensillum recordings or electroantennography (Ochieng and Hansson 1999; Chen and Kang 2000; Chen et al. 2004). This suggests that males are subject to sexual selection for locating (and possibly discriminating) females (Ritchie 1990). Chinese *Angaracris barabensis* grasshoppers rely on acoustic and visual cues to find mates and there is no sexual di erence in sensilla abundance (Chen and Kang

Fig. 4

thorax, legs, and wings (Jin et al. 2005; Guo et al. 2011; Wang et al. 2015; Zhang et al. 2018; Cui et al. 2019b; Yuan et al. 2019; Zhou et al. 2019; Li et al. 2020) implying broader functions. Some odorant receptors might be tuned to perceive pheromones, and in insects, odorant receptors are co-expressed with the protein SNMP1 only in pheromone-sensitive neurons (Cassau and Krieger 2021). Co-expression of odorant receptors and SNMP1 in sensilla basiconica and trichoidea of *S. gregaria* (Pregitzer et al. 2017) are responsible for detecting an aggregation pheromone and a putative

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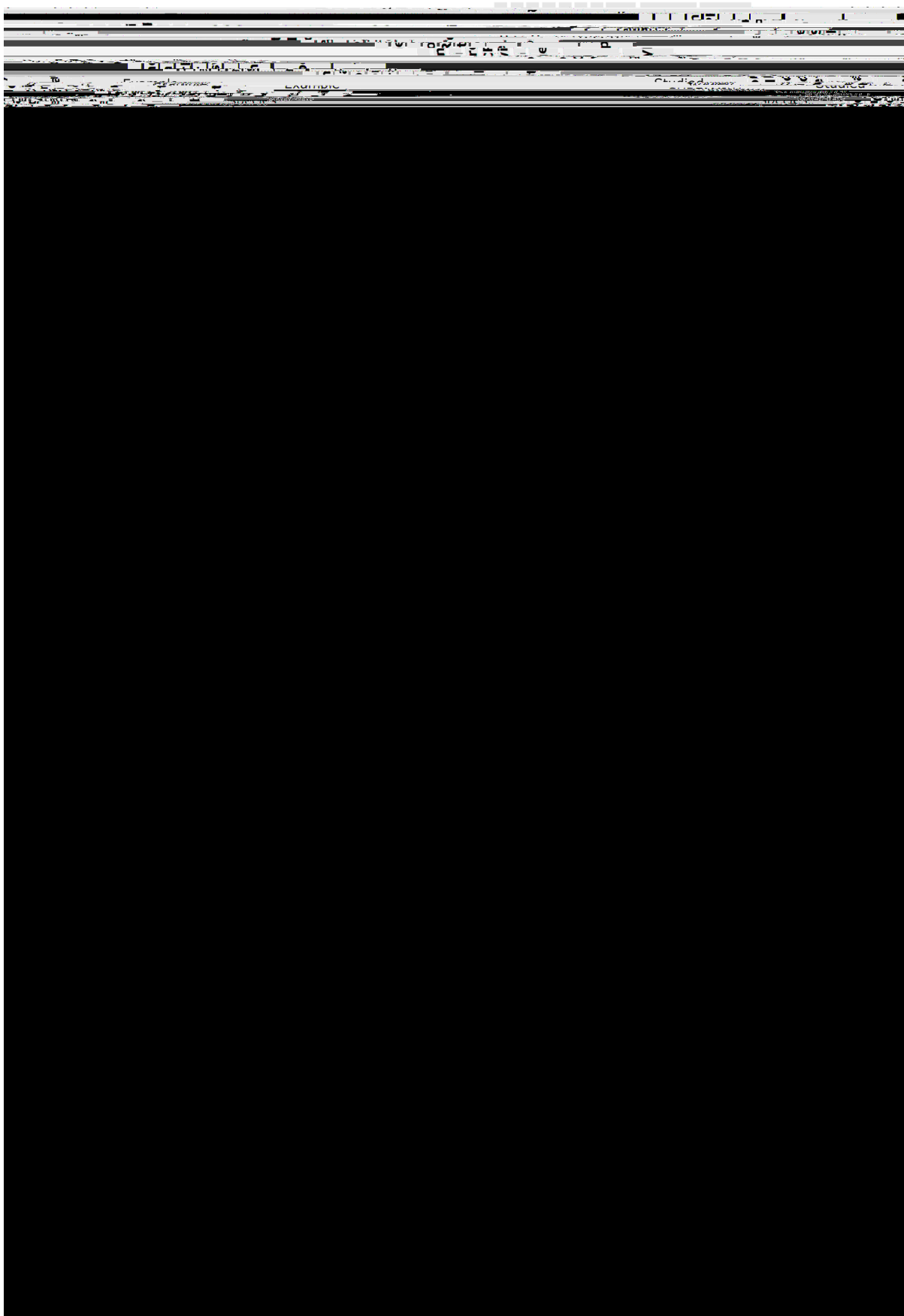
individuals may be reduced if plant-insect and insect-insect interactions are altered in natural populations of locusts by factors associated with climate change. Changes in irradiation intensity, temperature, CO_2 concentration, and humidity are known to affect the chemical composition (nutrients

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