Self-referent phenotype matching and its role in female mate choice in arthropods

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Abstract A growing body of empirical evidence shows that females of many animal species gain benefits by mating polyandrously, and often prefer to mate with novel males over previous mates. Although a female preference for novel males has been demonstrated for multiple animal taxa, the mechanisms used by females to discriminate between novel and previous mates remain largely unknown. However, recent studies suggest that in decorated crickets *Gryllodes sigillatus*, females actually imbue males with their own chemical cues, known as cuticular hydrocarbons (CHCs) during mating, and utilize chemosensory self-referencing to recognize recent mates. Here we review evidence that self-referent phenotype matching is a widespread mechanism of recognition in arthropods, and explore how CHCs are used to facilitate mate-choice decisions. There is substantial evidence that CHCs are used as recognition cues to discriminate between species, kin, sexes, mates, individuals, and self and non-self, and are used to facilitate mate-choice decisions in a wide range of arthropod taxa. There is also evidence that CHCs are often transferred between individuals during direct physical contact, including copulation. Chemosensory self-referencing via cuticular hydrocarbons could provide a simple, but reliable mechanism for identifying individuals from previous mating encounters. This mechanism does not require any specialized cognitive abilities because an individual's phenotype is always available for reference. Given the ubiquitous use of CHCs among arthropods, chemosensory self-referencing may be a widespread mechanism used by female arthropods to facilitate female mate-choice decisions and to enhance opportunities for polyandry [*Current Zoology* 59 (2): 239–248, 2013].

Keywords Polyandry, Chemical signals, Cuticular hydrocarbons, Sexual selection.

1 Introduction

The ability of an organism to recognize other individuals in the population can be of biological importance in many social interactions, especially those involving mate choice decisions (Greenfield, 2002). The evolution of animal communication systems has led, therefore, to complex visual, auditory and chemical cues that provide information about an individual's species, sex, genetic relatedness, mating status, social dominance, mate quality and individual identity (Mateo, 2004). Kin recognition is an important component of animal recognition systems, facilitating kin-biased behaviors such as inbreeding avoidance and altruism towards nestmates in social animals (Fletcher and Michener, 1987). In many species, learning and memory can be important for the formation of recognition templates, in which individuals learn the phenotypes of kin and use this memory as a template for comparison (Fletcher and Michener, 1987). However, in situations where constraints on learning and memory prohibit the formation of a recognition template, the use of self-referent phenotype matching may be used to identify closely related individuals (Mateo and Johnston, 2000). Self-referent phenotype matching requires only a simple, but reliable form of "on-line" processing in which an individual compares its own phenotype with that of the individuals with whom they interact (Holmes and Sherman, 1982; Lacy and Sherman, 1983; Sherman, 1991; Mateo and Johnston, 2000). Dubbed the "armpit effect" by Dawkins (1982), an individual in this context can compare its own 'scent' to the 'scent' of other individuals and modulate its behavior toward others based on the degree of similarity in the assessed phenotypes, such that the individual's own phenotype becomes the template for comparison.

In insects, chemical communication is an important mode of communication between individuals and it is often facilitated by cuticular hydrocarbons (Howard and Blomquist, 1982, 2005). Cuticular hydrocarbons (or CHCs) are lipid compounds that are present on the surface of the insect epicuticle, preventing desiccation and serving as a barrier to microorganisms (Lockey, 1988).

Besides providing these basic physiological functions, CHCs often play an integral role in insect chemical communication and have been demonstrated to function as important recognition cues facilitating species recognition, kin recognition and sex recognition in a variety of insect taxa (Howard and Blomquist, 1982, 2005). The chemical composition of CHCs consists of long carbon chains, making them ideal recognition cues with high chemical stability, low volatility and a diversity of structures allowing for significant variation in lipid composition (Howard and Blomquist, 1982; Blomquist et al., 1987; Howard and Blomquist, 2005). Indeed, self-referent phenotype matching via CHCs has been well examined as a mechanism to facilitate kin-biased behaviors in social insects (reviewed by Tsutsui, 2004).

In many animals, females mate more often than is necessary to ensure fertilization of their eggs, often mating polyandrously with several different males over their reproductive lifetime, and this behavior is especially prevalent in arthropods (Ridley, 1988). Indeed, empirical studies have demonstrated a female mating preference for novel males in a diverse range of taxa, including pseudoscorpions (Zeh et al., 1998), field crickets (Bateman, 1998; Ivy et al., 2005; Gershman, 2009), hide beetles (Archer and Elgar, 1999), dung flies (Hosken et al., 2003), and guppies (Eakley and Houde, 2004). While the possible ultimate explanations for polyandry have been widely examined among researchers (Zeh and Zeh, 1996, 1997; Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000; Slatyer et al., 2012), the proximate mechanisms underlying female mating decisions have received far less attention. What mechanisms are utilized by females over such a broad range of animal taxa to recognize previous mates, when cognitive constraints might limit their ability to learn the unique features of each of their mates over their reproductive lifetime?

Recent studies of the decorated cricket *Gryllodes sigillatus* have begun to provide some insight. Females of this species appear to imbue males with their own unique cuticular hydrocarbons (CHCs) during mating, and utilize chemosensory self-referencing to recognize recent mates in subsequent encounters (Ivy et al., 2005; Weddle et al., in press).　Here we review evidence that self-referent phenotype matching is a widespread mechanism of recognition in arthropods, and explore how cuticular hydrocarbons are used to facilitate mate-choice decisions across a wide range of arthropod taxa, including the decorated cricket. Given the ubiquitous use of CHCs as recognition cues among arthropods, we suggest that chemosensory self-referencing may be a widespread mechanism utilized by females to increase the diversity of their mating partners and maximize opportunities for polyandry.

2　Self-referent Phenotype Matching in Recognition Systems

Self-referent phenotype matching was first described as a mechanism of kin recognition in which an individual compares its own phenotype with that of other individuals as a means of assessing the degree of relatedness (Dawkins, 1982; Holmes and Sherman, 1982; Lacy and Sherman, 1983; Sherman, 1991; Mateo and Johnston, 2000). This mechanism of kin recognition would be favored in multiply mating species in which broods of mixed paternity are likely, offspring are widely dispersed, and individuals from the same brood may not meet again until adulthood (Hauber and Sherman, 2001). Under such circumstances, self-referent phenotype matching in the context of mate choice could provide a simple, but reliable means of kin discrimination to avoid the negative consequences of inbreeding.

Learning and memory are often invoked to explain recognition of nestmates, in which individuals learn the unique phenotypes of kin as a template for comparison (Fletcher and Michener, 1987). However, unlike social insects that are reared in large colonies with close genetic relatives, non-social insects exhibiting no parental care are more likely to have life history traits like those described above, in which individuals from the same brood may have very little exposure to close kin until adulthood. Self-referencing could be a simpler and more reliable mechanism of recognition than learning and memory for these animals, which, depending on dispersal patterns, may have limited exposure to kin or closely related individuals between the hatchling stage and the adult reproductive stage. In these systems, the phenotype of 'self' is always available for reference, and can be referred to during any interaction with another individual as a template for recognition (Hauber and Sherman, 2001).

While self-referent phenotype matching via chemosensory cues as a mechanism of kin recognition has been well examined in social insects (reviewed by Tsutsui, 2004), there is also evidence that some non-social insects can discriminate between chemosensory cues of 'self' and those of other individuals (Fellowes, 1998). Silk-spinning raspy crickets *Hyalogryllacris sp.* (Orthoptera: Gryllacrididae) are able to

discriminate between silk nests built by themselves and those of conspecifics by assessing and recognizing their own hydrocarbon chemical cues in the nesting silk (Lockwood and Rentz, 1996). Females of the cowpea seed beetle *Callosobruchus maculates* can distinguish between seeds bearing their own eggs and those bearing eggs of conspecific females (Ofuya and Agele, 1989). Several species of solitary Hymenopteran parasitoids have been shown to distinguish between hosts parasitized by themselves and those parasitized by conspecifics to avoid self-superparasitism (Hubbard et al., 1987; van Dijken et al., 1992; Godfray, 1994; Ueno, 1994). In the wasp *Nemeritis canescens*, this self-recognition is facilitated by a marking chemical produced by the Dufour's gland during oviposition on a host that the female is later able to recognize as her own (Hubbard et al., 1987).

Individual recognition has been shown to be important in establishing and maintaining dominance hierarchies in territorial invertebrates (Hazlett, 1969; Caldwell, 1979; Caldwell, 1985; Karavanich and Atema, 1998). However, invertebrate recognition of individuals in the context of female mate choice has received far less empirical attention. Earlier studies demonstrating female recognition of previous mates in arthropods have often invoked learning and memory of male traits, presumably chemical in nature, as the basis for individual mate recognition (Johnson, 1977; Linsenmair, 1985; Caldwell, 1992). While learning and memory may play an important role, especially for monogamous species (Linsenmair, 1985; Steiger et al., 2008b), they may not be as reliable a method of individual mate recognition for polyandrous species. Rather than a female learning and memorizing the traits of every male she mates with over her reproductive lifetime, a simpler and more reliable mechanism to recognize previous mates would be to simply assess a male for traces of her own chemical fingerprints.

3 Cuticular Hydrocarbons as Recognition Cues

Cuticular hydrocarbons as recognition cues contain a wide range of information content, with the hydrocarbon profiles of individuals varying specifically according to species, sex, age, colony, caste, and genetic background of the individual (Singer, 1998; Howard and Blomquist, 2005). Perhaps it is not surprising, therefore, that CHCs have been widely demonstrated as the chemical cues used for kin recognition in social insects,

with a significant genetic component to variation in the cues used to determine relatedness among nestmates (Howard et al., 1982; Howard, 1993; van Zweden and d'Ettorre, 2010). Cuticular hydrocarbons also act as cues for species and sex recognition, with many species exhibiting sex-specific variation in either the types or the relative proportions of cuticular compounds present (Howard and Blomquist, 1982, 2005). Extensive studies of *Drosophila* have demonstrated that CHCs can provide information about mate quality, and are important as sex pheromones for mate attraction and courtship, with a highly genetic component underlying variation in trait expression (reviewed by Ferveur, 2005). For example, studies of *Drosophila* suggest that certain male CHCs are under sexual selection by female choice (Hine et al., 2002; Chenoweth and Blows, 2003; Blows et al., 2004; Chenoweth and Blows, 2005; Petfield et al., 2005; Foley et al., 2007). Extensive research on the *Drosophila serrata* species complex occurring in sympatry with *Drosophila birchii* indicates a complex interaction between sexual selection and natural selection on variation in male and female CHC profiles used in mate choice and mate recognition, and demonstrate the evolutionary importance of these chemical cues for the maintenance of reproductive isolation (Higgie et al., 2000; Blows, 2002; Blows and Higgie, 2002, 2003; Howard et al., 2003; Skroblin and Blows, 2006; Higgie and Blows, 2007).

In addition to providing information about an individual's species, sex and genetic relatedness, CHCs can also provide information about an individual's mating status, thereby facilitating mate-choice decisions (reviewed by Thomas, 2011). Cuticular hydrocarbons have been shown to signal the fertility status of reproductives to other colony members in several species of social Hymenoptera (Peeters and Liebig, 2009; Liebig, 2010). Female mosquitoes and Dawson's burrowing bees, show significant changes in the proportions of specific CHC compounds in the cuticle after mating, and application of extracts from mated to unmated females causes a reduction in rates of female insemination by males (Polerstock et al., 2002; Simmons et al., 2003). In *Drosophila melanogaster*, previously mated females produce predominantly male-specific CHC substances in the presence of courting males, thereby mimicking the male 'scent,' which seems to function as a male anti-aphrodisiac (Scott, 1986; Scott et al., 1988; Scott and Jackson, 1990). Friberg (2006) showed that *Drosophila* males can distinguish the CHCs of mated females from virgin females and may use these cues to deter-

mine the risk of sperm competition. The chemical composition of CHC profiles for cooperative breeding *Nicrophorus* burying beetles changes during the breeding cycle, and is used to distinguish breeding partners from conspecific intruders on a breeding carcass (Steiger et al., 2007; Scott et al., 2008; Steiger et al., 2008a; Steiger et al., 2009).

There is also evidence that CHCs can be transferred between individuals through direct physical contact. Several studies have shown that living in mixed species groups results in a blended CHC profile for all individuals, in which compounds distinct to each species can be found in blends on all individuals from the group. After species have been separated for some time, the distinct CHC profiles are reestablished, indicating that CHCs are transferred among individuals via physical contact during group living. This phenomenon has been documented for *Drosophila* (Coyne et al., 1994; Blows and Allan, 1998), ants (Vienne et al., 1995) termites (Vauchot et al., 1996; Vauchot et al., 1997), and cockroaches (Everaerts et al., 1997). A study of carpenter ants, *Camponotus fellah*, suggests that the 'Gestalt' colony CHC profile used as a mechanism of nestmate recognition is maintained through a continual exchange of CHCs between individuals during grooming and trophallaxis (Soroker et al., 1995; Boulay et al., 2000).

CHC compounds can also be transferred by physical contact during copulation. Female cockroaches *Nauphoeta cinerea* discriminate against potentially sperm-limited males that have had multiple female mating partners, and this effect was also observed when epicuticular rubbings from multiple females were applied to virgin males, indicating CHC compounds are transferred during copulation (Harris and Moore, 2005). Male field crickets *Teleogryllus oceanicus* respond to the perceived risk of sperm competition by adjusting their ejaculate allocation in response to the number of distinct CHC extracts from individual males present on females (Thomas and Simmons, 2009). *Drosophila melanogaster* show reciprocal variation in CHCs due to mechanical transfer of compounds between males and females during copulation (Scott, 1986; Scott et al., 1988; Everaerts et al., 2010). Male *Drosophila* transfer the male-predominant cuticular hydrocarbon 7-tricosene to females during mating, and the amount detected on females corresponds to an approximately equivalent, concurrent decrease of this compound found on males, consistent with male-to-female transfer through direct physical contact (Scott, 1986). Similarly, the female-specific courtship pheromone 7,11-heptacosadiene is

transferred from females to males during copulation, and can be detected in the cuticular extracts of males after mating (Scott and Richmond, 1987). If females are capable of transferring their own unique CHCs to males during the physical act of copulation (Scott and Richmond, 1987; Harris and Moore, 2005), and are later able to recognize these cues via self-referencing in subsequent encounters, this could provide a simple but reliable mechanism by which females could identify previous mating partners.

4 Polyandry and Self-referencing in the Decorated Cricket

The decorated cricket *Gryllodes sigillatus* occurs worldwide, in both tropical and subtropical regions and its occurrence is often associated with human habitation (Smith and Thomas, 1988). Male decorated crickets initiate courtship after establishing antennal contact with a female. Courtship consists of a characteristic courtship song, accompanied by rhythmic lateral movements of the body (Loher and Dambach, 1989; Zuk and Simmons, 1997). If a female is sexually responsive, she will mount the male dorsally, and the male will attempt to secure her subgenital plate with his epiphallus, a requirement for spermatophore transfer (Sakai et al., 1991). Copulation is completed with the successful transfer of a spermatophore, which in *G. sigillatus* consists of a small, sperm-containing ampulla surrounded by a large gelatinous mass known as the spermatophylax that the females consumes after mating (Sakaluk, 1984, 1985, 1987, 2000). During mating, the male and female remain in direct physical contact until the male successfully transfers a spermatophore (Fig. 1), a process that

Fig. 1   A mating pair of the decorated cricket *Gryllodes sigillatus*
The female's (above) ventral side remains in direct physical contact with the male's (below) dorsum for 2–4 minutes during copulation. Photograph by Scott Sakaluk.

typically requires about 2-4 minutes (Sakaluk, 1987).

Field studies of this species have shown that females often mate multiply over the course of an evening, but rarely with the same male twice (Sakaluk et al., 2002). Multiple mating provides fitness benefits to females, but these benefits are only observed when females mate polyandrously (Sakaluk et al., 2002; Ivy and Sakaluk, 2005; Ivy, 2007). Female *G. sigillatus* preferentially mate with novel mating partners over previous mating partners in mate-choice trials (Ivy et al., 2005). This result suggests that females rely on some underlying mechanism to recognize previous mates. Indeed, we have shown experimentally that female *G. sigillatus* avoid mating with a male that has previously mated with her inbred sister (Ivy et al., 2005). In these experiments, "familiar" males were mated to an inbred sister of the focal female 24-hours prior to mate-choice trials. Subsequently, the focal female was allowed to choose between the "familiar" male and a novel male, and showed a marked preference for the novel male (Fig. 2). Females had no prior experience with either male, and males were of comparable sexual experience at the time of experimental trials. Individuals within a genetic line were presumed to share a high degree of phenotypic similarity in the chemical cues that might be used in individual recognition (coefficient of inbreeding, $F=0.5$). Our results suggest that the focal female perceived the chemical cue left on the male by her inbred sister as her own, and consequently identified the familiar male as a previous mating partner. Because females had no prior experience with either male, their preference for the

novel males cannot be attributed to their having learned specific features of 'familiar' males.

To further distinguish between learning and self-referencing, we conducted a second experiment to determine if females rely on male-specific cues to identify previous mating partners. Females were allowed to choose between the inbred brother of a male she had mated with 24-hours prior to mate-choice trials ("familiar male"), or a novel male of comparable sexual experience. Experimental females showed no significant mating preference for either of the males in these trials (Ivy et al., 2005; Fig. 2). Thus, it does not appear that females use male-specific cues (via learning and memory) to recognize previous mating partners. Instead, the results suggest that females imbue males with their own unique chemical cues during mating, and are later able to recognize these cues in subsequent encounters.

We have recently demonstrated that cuticular hydrocarbons are the underlying chemical cues used by *G. sigillatus* females to facilitate recognition and discrimination against previous mating partners (Weddle et al., in press). Utilizing gas chromatography-mass spectrometry (GC-MS), we analyzed hexane extracts of CHCs for females from nine isofemale lines, identifying 15 distinct hydrocarbons ranging from 33 to 41 carbons in length and consisting of branched alkanes, alkatrienes and alkenes (Weddle et al., in press). Comparison of 13 of these hydrocarbons across genetic lines revealed substantial genetic variance in the cuticular hydrocarbon profiles of females, with an average heritability (± 1 *SE*) of 0.978 ± 0.008, indicating that they can provide phenotypically unique chemical signatures that would allow discrimination of 'self' from the cues of other individuals they encounter.

In a second experiment, we used SPME (Solid Phase Micro Extraction) fibers to test male CHCs before and after copulation. The advantage of using SPME in behavioral studies is that it is capable of accurately sampling small quantities of CHCs directly from the epicuticular surface of a live animal without the use of harmful solvents (Moneti et al., 1997; Augusto and Valente, 2002). We were able, therefore, to directly sample the dorsal surface of the male which comes into direct contact with the female during mating (Fig.1). The CHC profiles of males and females show the same distinct CHC compounds, with marked sexual dimorphism due to variation in the proportions of these compounds (Weddle et al., 2012). The results of the SPME study demonstrated that the CHC profiles of males become more similar to those of females after mating, indicating
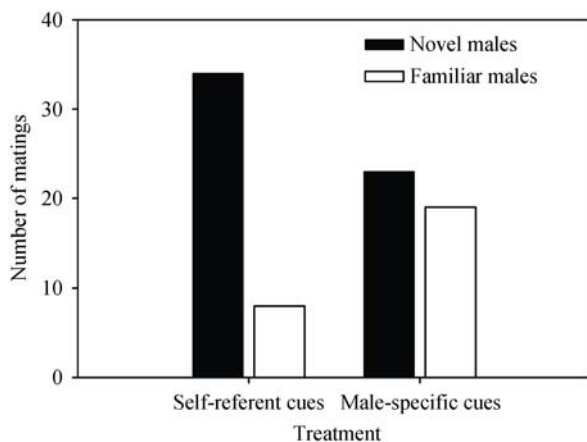

Fig. 2 **The mating frequency of *G. sigillatus* females that mated with (i) a novel male and a male with whom her inbred sister had previously mated (self-referent cues) and (ii) a novel male and the inbred sibling of a male with whom she had already mated (male-specific cues)**
Redrawn from Ivy et al., 2005.

that CHCs are physically transferred from females to males during copulation (Weddle et al., in press). Compounds that were more abundant in females tended to increase in mated males, while compounds that were less abundant in females showed a relative decrease in males after mating (Fig. 3).

In a third experiment, we performed a behavioral bioassay, in which we externally applied female CHCs to males to test whether CHCs directly affect female mate-choice behavior. In mate-choice trials, we found that females showed an aversion to mating with males bearing chemical cues similar to their own (Weddle et al., in press; Fig. 4). Lastly, we tested the hypothesis that chemosensory self-referencing is used as a mechanism of kin recognition to facilitate pre-copulatory inbreeding avoidance. In mate-choice trials, females showed no significant mating preference for unrelated males over sibling males, suggesting that discrimination against previous mates via self-referencing is not simply an incidental consequence of a mechanism that evolved to facilitate inbreeding avoidance (Weddle et al., in press).

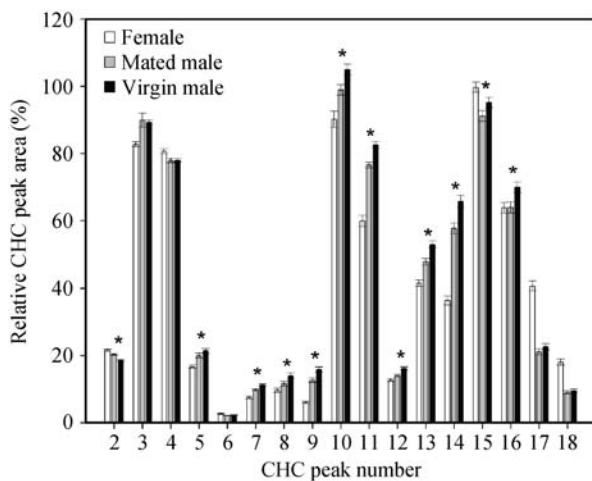The environment can be an important source of within-population variance in CHCs (Rundle et al., 2005),
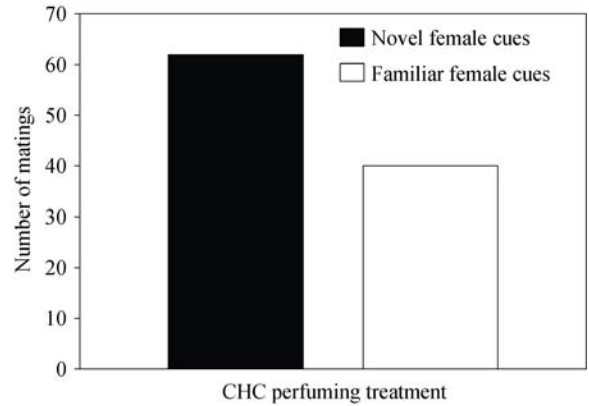

Fig. 4   The mating frequency of focal females that were allowed to choose between two virgin males from the same genetic line (different from that of the female)

Males were externally treated with either: (i) CHC extracts from females of the same age, but from a different genetic line than the focal female (novel female cues); or (ii) CHC extracts from sibling females of the same age and genetic line as the focal female (familiar female cues). Focal females mated significantly more often with 'novel' males bearing the CHC cues of unrelated, inbred females than with 'familiar' males bearing the cues of inbred sisters (χ^2 test for equal proportions: $\chi^2 = 4.75$, $P = 0.029$, $n = 102$).

Fig. 3   Mean ($\pm$ *SE*) relative peak area for each CHC peak for *G. sigillatus* virgin females, mated males and virgin males as detected by SPME. Each peak number corresponds to a specific CHC compound

Comparisons of each male CHC peak before and after mating showed significant differences (indicated by asterisks) between virgin and mated males in 12 out of 18 CHCs detected and that the CHC profiles of mated males becomes more similar to that of females for all but one of these compounds (peak 15) (Repeated Measures MANOVA, Bonferroni adjusted $\alpha = 0.0029$). Note that SPME detected 3 additional CHC peaks not detected in solvent extracts. Peak 1 was used to standardize the remaining 17 peaks and was therefore not included in the analysis. Redrawn from Weddle et al., in press.

and there is evidence that dietary hydrocarbons are incorporated into the cuticular lipids of many insects from the food they consume (Blomquist and Jackson, 1973). Therefore, in another recent study, we manipulated the nutritional environment of females over their developmental lifetime to determine the extent to which variation in the CHCs of this species is influenced by diet. We found a marginal effect of diet on the overall quantity of all female CHC compounds, suggesting perhaps, some phenotypic plasticity for these cues (Weddle et al., 2012). However, we found no significant genotype-by-environment interaction for variation in female CHCs, suggesting that females can allocate sufficient resources toward the production or maintenance of optimal levels of CHC expression for reliable signal content, regardless of genetic background (Weddle et al., 2012). The results of these findings indicate that while the environment may affect the relative strength of the CHC signal of 'self' for females, it does not appear to influence signal content, as overall the signal remains relatively intact over both environments and genotypes, making CHCs reliable cues for the recognition of 'self'.

It remains unclear how long CHC cues last after they are transferred to males during mating, which could influence the efficacy of these cues for mate-choice decisions. We do know from previous work that *G. sigillatus* females show behavioral discrimination against

previous mates for at least 24–28 hours after initial copulation (Ivy et al., 2005). Any benefits to self-referencing are going to depend, of course, on the frequency with which females encounter previous mates. Although we cannot state with any certainty the probability that a female will encounter a previous mate in nature, lifetime measures of male and female mating success of marked individuals in a large outdoor enclosure (Sakaluk et al. 2002) suggest that it can be quite high, at least over the course of the same night or two consecutive nights. Sakaluk et al. (2002) reported that individuals of both sexes typically aggregate in large clusters in only a small minority of the shelters that are available, and that they remain in these shelters throughout the majority of the day. Nearly 30% of all nightly matings recorded in this study involved females mating for the 2nd, 3rd or 4th time in a night, but interestingly, on only two occasions did a female ever mate with the same male twice. Females tend to travel an average distance of two meters over consecutive nights, moving over greater distances than males of this species (Sakaluk, 1987). Therefore, a brief efficacy may be all that is required to maintain recognition of 'self' if females are likely to move to a new area over the course of several days, reducing the likelihood of encountering previous mates during that time.

5   Conclusions

An inherent difficulty in demonstrating self-referent phenotype matching as a mechanism of recognition is that evidence is often circumstantial (Hare et al., 2003). It can prove logistically difficult to alter an individual's phenotype, or change its experience with its own phenotype, without potentially altering the individual's normal behavior (Hauber and Sherman, 2001). Most studies of self-referent phenotype matching for kin recognition, therefore, rely on using naïve experimental subjects that have had no prior experience with close kin or relatives. Prior to recent studies involving decorated crickets (Ivy et al., 2005; Weddle et al., in press), the only evidence for chemosensory self-referencing via CHCs to facilitate mate-choice decisions in arthropods has come from studies of kin recognition as a mechanism of inbreeding avoidance. Several studies have demonstrated inbreeding avoidance via kin discrimination in arthropods implying chemosensory self-referencing via CHCs as the most likely proximate mechanism (Simmons, 1989; Enigl and Schausberger, 2004; Lihoreau et al., 2007; Metzger et al., 2010). However, few studies have demonstrated the use of CHCs experimentally through the

use of behavioral bioassays. Chemosensory self-referencing via CHCs has been demonstrated as a mechanism of kin recognition to facilitate inbreeding avoidance in the field cricket *Gryllus bimaculatus* (Simmons, 1990), the non-social, parasitic wasp *Venturia canescens* (Metzger et al., 2010), and the gregarious cockroach *Blattella germanica* (Lihoreau and Rivault, 2009). Ali and Tallamy (2010) demonstrated that female spotted cucumber beetles *Diabrotica undecimpunctata howardi* show a mating preference for males that have a CHC profile dissimilar to their own as a mechanism for choosing immunocompatible mates, and implied that females may be using their own CHC profile as a standard for comparison. We suggest the need for further studies designed to test the mechanism of chemosensory self-referencing through the use of behavioral bioassays that directly assess the effects of CHCs on female mate-choice decisions.

Although the evolutionary significance of polyandry has been widely examined both theoretically and empirically, the proximate mechanisms by which female mate-choice decisions are facilitated remain unclear for many mating systems. To our knowledge, the results of Ivy et al. (2005) and (Weddle et al., in press) are the first to describe a possible mechanism by which female arthropods recognize and discriminate against previous mating partners. Chemosensory self-referencing via cuticular hydrocarbons could provide a simple, but reliable mechanism for identifying individuals from previous mating encounters. Indeed, because an individual's phenotype is always available for reference, this mechanism would not require any specialized cognitive abilities. Instead, it would simply require a female to assess a male for traces of her own unique chemical profile during any mating encounter (Hauber and Sherman, 2001). Given the ubiquitous use of CHCs as recognition cues among arthropods (Howard and Blomquist, 1982, 2005), chemosensory self-referencing may be a widespread mechanism used by female arthropods to facilitate female mate-choice decisions and maximize opportunities for polyandry.

References

Ali JG, Tallamy DW, 2010. Female spotted cucumber beetles use own cuticular hydrocarbon signature to choose immunocompatible mates. Anim. Behav. 80: 9–12.

Archer MS, Elgar MA, 1999. Female preference for multiple partners: Sperm competition in the hide beetle *Dermestes maculatus* (DeGeer). Anim. Behav. 58: 669–675.

Arnqvist G, Nilsson T, 2000. The evolution of polyandry: Multiple mating and female fitness in insects. Anim. Behav. 60: 145–164.

Augusto F, Valente ALP, 2002. Applications of solid-phase microextraction to chemical analysis of live biological samples. Trend. Anal. Chem. 21: 428–438.

Bateman PW, 1998. Mate preference for novel partners in the cricket *Gryllus bimaculatus*. Ecol. Entomol. 23: 473–475.

Blomquist GJ, Jackson LL, 1973. Incorporation of labelled dietary *n*-alkanes into cuticular lipids of the grasshopper *Melanoplus sanguinipes*. J. Insect Physiol. 19: 1639-1647.

Blomquist GJ, Nelson DR, de Renoblades M, 1987. Chemistry, biochemistry and physiology of insect cuticular lipids. Arch. Insect Biochem. Physiol. 6: 227–265.

Blows MW, 2002. Interaction between natural and sexual selection during the evolution of mate recognition. Proc. R. Soc. Lond. B 269: 1113–1118.

Blows MW, Allan RA, 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. Am. Nat. 152: 826–837.

Blows MW, Higgie M, 2002. Evolutionary experiments on mate recognition in the *Drosophila serrata* species complex. Genetica 116: 239–250.

Blows MW, Higgie M, 2003. Genetic constraints on the evolution of mate recognition under natural selection. Am. Nat. 161: 240–253.

Blows MW, Chenoweth SF, Hine E, 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am. Nat. 163: 329–340.

Boulay R, Hefetz A, Soroker V, Lenoir A, 2000. *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. Anim. Behav. 59: 1127–1133.

Caldwell RL, 1979. Cavity occupation and defensive behaviour in the stomatopod *Gonodactylus festae*: Evidence for chemically mediated individual recognition. Anim. Behav. 27: 194-201.

Caldwell RL, 1985. A test of individual recognition in the stomatopod *Gonodactylus festate*. Anim. Behav. 33: 101–106.

Caldwell RL, 1992. Recognition, signaling and reduced aggression between former mates in a stomatopod. Anim. Behav. 44: 11–19.

Chenoweth SF, Blows MW, 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. Evolution 57: 2326–2334.

Chenoweth SF, Blows MW, 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. Am. Nat. 165: 281–289.

Coyne JA, Crittenden AP, Mah K, 1994. Genetics of a pheromonal difference contributing to reproductive isolation in *Drosophila*. Science 265: 1461–1464.

Dawkins R, 1982. The Extended Phenotype. New York, NY: Oxford University Press Inc.

Eakley AL, Houde AE, 2004. Possible role of female discrimination against 'redundant' males in the evolution of colour pattern polymorphism in guppies. Proc. R. Soc. Lond. B 271 (Suppl 5): S299–301.

Enigl M, Schausberger P, 2004. Mate choice in the predaceous mite *Phytoseiulus persimilis*: Evidence of self-referent phenotype matching? Entomol. Exp. Appl. 112: 21-28.

Everaerts C, Farine JP, Brossut R, 1997. Changes of species specific cuticular hydrocarbon profiles in the cockroaches *Nauphoeta cinerea* and *Leucophaea maderae* reared in heterospecific groups. Entomol. Exp. Appl. 85: 145–150.

Everaerts C, Farine J-P, Cobb M, Ferveur J-F, 2010. *Drosophila* cuticular hydrocarbons revisited: Mating status alters cuticular profiles. PLoS One 5: 1–12.

Fellowes MDE, 1998. Do non-social insects get the (kin) recognition they deserve? Ecol. Entomol. 23: 223–227.

Ferveur J-F, 2005. Cuticular hydrocarbons: Their evolution and roles in *Drosophila* pheromonal communication. Behav. Genet. 35: 279–295.

Foley B, Chenoweth SF, Nuzhdin SV, Blows MW, 2007. Natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. Genetics 175: 1465–1477.

Friberg U, 2006. Male perception of female mating status: Its effect on copulation duration, sperm defence and female fitness. Anim. Behav. 72: 1259–1268.

Gershman SN, 2009. Postcopulatory female choice increases the fertilization success of novel males in the field cricket *Gryllus vocalis*. Evolution 63: 67–72.

Godfray HCJ, 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton, NJ: Princeton University Press.

Greenfield MD, 2002. Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication. New York, NY: Oxford University Press.

Hare JF, Sealy SG, Underwood TJ, Ellison KS, Stewart RLM, 2003. Evidence of self-referent phenotype matching revisited: Airing out the armpit effect. Anim. Cogn. 6: 65-68.

Harris WE, Moore PJ, 2005. Female mate preference and sexual conflict: Females prefer males that have had fewer consorts. Am. Nat. 165: S64–S71.

Hauber ME, Sherman PW, 2001. Self-referent phenotype matching: Theoretical considerations and empirical evidence. Trends Neurosci. 24: 609-616.

Hazlett BA, 1969. "Individual" recognition and agonistic behaviour in *Pagurus bernhardus*. Nature 222: 268–269.

Higgie M, Blows MW, 2007. Are traits that experience reinforcement also under sexual selection? Am. Nat. 170: 409–420.

Higgie M, Chenoweth S, Blows MW, 2000. Natural selection and the reinforcement of mate recognition. Science 290: 519–521.

Hine E, Lachish S, Higgie M, Blows MW, 2002. Positive genetic correlation between female preference and offspring fitness. Proc. R. Soc. Lond. B 269: 2215–2219.

Holmes WG, Sherman PW, 1982. The ontogeny of kin recognition in two species of ground squirrels. Am. Zool. 22: 491–517.

Hosken DJ, Martin OY, Born J, Huber F, 2003. Sexual conflict in *Sepsis cynipsea*: Female reluctance, fertility and mate choice. J. Evol. Biol. 16: 485–490.

Howard RW, 1993. Cuticular hydrocarbons and chemical com-

munication. In: Stanley-Samuelson DW, Nelson DR ed. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 179–226.

Howard RW, Blomquist GJ, 1982. Chemical ecology and biochemistry of insect hydrocarbons. Annu. Rev. Entomol. 27: 149–172.

Howard RW, Blomquist GJ, 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu. Rev. Entomol. 50: 371–393.

Howard RW, Jackson LL, Banse H, Blows MW, 2003. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: Identification and role in mate choice in *D. serrata*. J. Chem. Ecol. 29: 961–976.

Howard RW, McDaniel CA, Nelson DR, Blomquist GJ, Gelbaum LT et al., 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species- and caste-recognition cues. J. Chem. Ecol. 8: 1227–1239.

Hubbard SF, Marris G, Reynolds A, Rowe GW, 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. J. Anim. Ecol. 56: 387–401.

Ivy TM, 2007. Good genes, genetic compatibility and the evolution of polyandry: Use of the diallel cross to address competing hypotheses. J. Evol. Biol. 20: 479-487.

Ivy TM, Sakaluk SK, 2005. Polyandry promotes enhanced offspring survival in decorated crickets. Evolution 59: 152–159.

Ivy TM, Weddle CB, Sakaluk SK, 2005. Females use self-referent cues to avoid mating with previous mates. Proc R. Soc. B 272: 2475–2478.

Jennions MD, Petrie M, 2000. Why do females mate multiply? A review of the genetic benefits. Biol. Rev. Camb. Philos. Soc. 75: 21–64.

Johnson VR, Jr., 1977. Individual recognition in the banded shrimp *Stenopus hispidus* (Olivier). Anim. Behav. 25: 418–428.

Karavanich C, Atema J, 1998. Individual recognition and memory in lobster dominance. Anim. Behav. 56: 1553–1560.

Lacy RC, Sherman PW, 1983. Kin recognition by phenotype matching. Am. Nat. 121: 489–512.

Liebig J, 2010. Hydrocarbon profiles indicate fertility and dominance status in ant, bee, and wasp colonies. In: Blomquist GJ, Bagnères A-G ed. Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology. Cambridge: Cambridge University Press, 254–281.

Lihoreau M, Rivault C, 2009. Kin recognition via cuticular hydrocarbons shapes cockroach social life. Behav. Ecol. 20: 46–53.

Lihoreau M, Zimmer C, Rivault C, 2007. Kin recognition and incest avoidance in a group-living insect. Behav. Ecol. 18: 880–887.

Linsenmair KE, 1985. Individual and family recognition in subsocial arthropods, in particular in the desert isopod *Hemilepistus reaumuri*. Fortschr. Zool. 31: 411–436.

Lockey KH, 1988. Lipids of the insect cuticle: origin, composition and function. Comp. Biochem. Physiol. B. 89: 595–645.

Lockwood JA, Rentz DCF, 1996. Nest construction and recognition in a gryllacridid: the discovery of pheromonally mediated autorecognition in an insect. Aust. J. Zool. 44: 129–141.

Loher W, Dambach M, 1989. Reproductive behavior. In: Huber F, Moore TE, Loher W ed. Cricket Behavior and Neurobiology.

Ithaca, NY: Cornell University Press, 43–82.

Mateo JM, 2004. Recognition systems and biological organization: The perception component of social recognition. Ann. Zool. Fennici 41: 729–745.

Mateo JM, Johnston RE, 2000. Kin recognition and the 'armpit effect': Evidence of self-referent phenotype matching. Proc. R. Soc. Lond. B 267: 695–700.

Metzger M, Bernstein C, Hoffmeister TS, Desouhant E, 2010. Does kin recognition and sib-mating avoidance limit the risk of genetic incompatibility in a parasitic wasp? PLoS ONE 5: e13505.

Moneti G, Dani FR, Pieraccini G, Turillazzi S, 1997. Solid-phase microextraction of insect epicuticular hydrocarbons for gas chromatographic/mass spectrometric analysis. Rapid Commun. Mass Spectrom. 11: 857–862.

Ofuya T, Agele S, 1989. Ability of ovipositing *Callosobruchus maculatus* females to discriminate between seeds bearing their own eggs and those bearing eggs of other females. Ecol. Entomol. 14: 243–246.

Peeters C, Liebig J, 2009. Fertility signaling as a general mechanism of regulating reproductive division of labor in ants. In: Gadau J, Fewel J ed. Organization of Insect Societies: From Genome to Socio-Complexity. Cambridge: Harvard University Press, 220–242.

Petfield D, Chenoweth SF, Rundle HD, Blows MW, 2005. Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. Proc. Natl. Acad. Sci. 102: 6045–6050.

Polerstock AR, Eigenbrode SD, Klowden MJ, 2002. Mating alters the cuticular hydrocarbons of female *Anopheles gambiae* sensu stricto and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 39: 545–552.

Ridley M, 1988. Mating frequency and fecundity in insects. Biol. Rev. Camb. Philos. Soc. 63: 509–549.

Rundle HD, Chenoweth SF, Doughty P, Blows MW, 2005. Divergent selection and the evolution of signal traits and mating preferences. PLoS Biol. 3: e368.

Sakai M, Toaoda Y, Mori K, Fujino M, Ohta C, 1991. Copulation sequence and mating termination in the male cricket *Gryllus bimaculatus* DeGeer. J. Insect Physiol. 37: 599–615.

Sakaluk SK, 1984. Male crickets feed females to ensure complete sperm transfer. Science 223: 609–610.

Sakaluk SK, 1985. Spermatophore size and its role in the reproductive behaviour of the cricket *Gryllodes supplicans* (Orthoptera: Gryllidae). Can. J. Zool. 63: 1652–1656.

Sakaluk SK, 1987. Reproductive behaviour of the decorated cricket *Gryllodes supplicans* (Orthoptera: Gryllidae): Calling schedules, spatial distribution, and mating. Behaviour 100: 202–225.

Sakaluk SK, 2000. Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. Proc. R. Soc. Lond. B 267: 339–343.

Sakaluk SK, Schaus JM, Eggert AK, Snedden WA, Brady PL, 2002. Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. Evolution 56: 1999–2007.

Scott D, 1986. Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. Proc. Natl. Acad. Sci. USA 83: 8429–8433.

Scott D, Richmond RC, 1987. Evidence against an antiaphrodisiac role for cis-vaccenyl acetate in *Drosophila melanogaster*. J. Insect Physiol. 33: 363–369.

Scott D, Jackson LL, 1990. The basis for control of post-mating sexual attractiveness by *Drosophila melanogaster* females. Anim. Behav. 40: 891.

Scott D, Richmond RC, Carlson DA, 1988. Pheromones exchanged during mating: a mechanism for mate assessment in *Drosophila*. Anim. Behav. 36: 1164–1173.

Scott MP, Madjid K, Orians CM, 2008. Breeding alters cuticular hydrocarbons and mediates partner recognition by burying beetles. Anim. Behav. 76: 507–513.

Sherman PW, 1991. Multiple mating and kin recognition by self-inspection. Ethol. Sociobiol. 12: 377–386.

Simmons LW, 1989. Kin recognition and its influence on mating preferences of the field cricket *Gryllus bimaculatus* (de Geer). Anim. Behav. 38: 68–77.

Simmons LW, 1990. Pheromonal cues for the recognition of kin by female field crickets *Gryllus bimaculatus*. Anim. Behav. 40: 192–195.

Simmons LW, Alcock J, Reeder A, 2003. The role of cuticular hydrocarbons in male attraction and repulsion by female Dawson's burrowing bee *Amegilla dawsoni*. Anim. Behav. 66: 677–685.

Singer TL, 1998. Roles of hydrocarbons in the recognition systems of insects. Am. Zool. 38: 394–405.

Skroblin A, Blows MW, 2006. Measuring natural and sexual selection on breeding values of male display traits in *Drosophila serrata*. J. Evol. Biol. 19: 35–41.

Slatyer RA, Mautz BS, Backwell PRY, Jennions MD, 2012. Estimating genetic benefits of polyandry from experimental studies: A meta-analysis. Biol. Rev. 87: 1–33.

Smith RL, Thomas WB, 1988. Southwestern distribution and habitat ecology of *Gryllodes supplicans*. Bull. Entomol. Soc. Am. 34: 186–190.

Soroker V, Vienne C, Hefetz A, 1995. Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera, Formicidae). J. Chem. Ecol. 21: 365–378.

Steiger S, Peschke K, Francke W, Müller JK, 2007. The smell of parents: Breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. Proc R. Soc. B 274: 2211–2220.

Steiger S, Peschke K, Müller JK, 2008a. Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: The chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*? Behav. Ecol. Sociobiol. 62: 1053–1060.

Steiger S, Franz R, Eggert AK, Müller JK, 2008b. The Coolidge effect, individual recognition and selection for distinctive cuticular signatures in a burying beetle. Proc R. Soc. B 275: 1831–1838.

Steiger S, Whitlow S, Peschke K, Müller JK, 2009. Surface chemicals inform about sex and breeding status in the biparental burying beetle *Nicrophorus vespilloides*. Ethology 115: 178–185.

Thomas ML, 2011. Detection of female mating status using chemical signals and cues. Biol. Rev. Camb. Philos. Soc. 86: 1–13.

Thomas ML, Simmons LW, 2009. Male-derived cuticular hydrocarbons signal sperm competition intensity and affect ejaculate expenditure in crickets. Proc. R. Soc. B 276: 383–388.

Tsutsui ND, 2004. Scents of self: the expression component of self/non-self recognition systems. Ann. Zool. Fenn. 41: 713–727.

Ueno T, 1994. Self-recognition by the parasitic wasp *Itoplectis naranyae* (Hymenoptera: Ichneumonidae). Oikos 70: 333–339.

van Dijken MJ, van Stratum P, van Alphen JJM, 1992. Recognition of individual-specific marked parasitized hosts by the solitary parasitoid *Epidinocarsis lopezi*. Behav. Ecol. Sociobiol. 30: 77–82.

van Zweden JS, d'Ettorre P, 2010. Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist GJ, Bagnères A-G ed. Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology. Cambridge: Cambridge University Press, 222–243.

Vauchot B, Provost E, Clement JL, 1997. Pattern of recovery of species-specific cuticular hydrocarbon mixtures by *Reticulitermes santonensis* and *Reticulitermes lucifugus grassei* after being removed from a mixed group - is the acquisition of allospecific hydrocarbons reversible? Arch. Insect Biochem. Physiol. 35: 237–259.

Vauchot B, Provost E, Bagnères AG, Clement JL, 1996. Regulation of the chemical signatures of two termite species *Reticulitermes santonensis* and *Reticulitermes lucifugus grassei*, living in mixed experimental colonies. J. Insect Physiol. 42: 309–321.

Vienne C, Soroker V, Hefetz A, 1995. Congruency of hydrocarbon patterns in heterospecific groups of ants: transfer and/or biosynthesis? Insect. Soc. 42: 267–277.

Weddle CB, Mitchell C, Bay SK, Sakaluk SK, Hunt J, 2012. Sex-specific genotype-by-environment interactions for cuticular hydrocarbon expression in decorated crickets *Gryllodes sigillatus*: Implications for the evolution of signal reliability. J. Evol. Biol. 25: 2112–2125.

Weddle CB, Steiger S, Hamaker CG, Ower GD, Mitchell C et al., in press. Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets: A potentially universal mechanism facilitating polyandry in insects. Ecol. Lett.

Zeh JA, Zeh DW, 1996. The evolution of polyandry I: Intragenomic conflict and genetic incompatibility. Proc. R. Soc. Lond. B 263: 1711–1717.

Zeh JA, Zeh DW, 1997. The evolution of polyandry II: Post-copulatory defenses against genetic incompatibility. Proc. R. Soc. Lond. B 264: 69–75.

Zeh JA, Newcomer SD, Zeh DW, 1998. Polyandrous females discriminate against previous mates. Proc. Natl. Acad. Sci. USA 95: 13732– 13736.

Zuk M, Simmons LW, 1997. Reproductive strategies of the crickets (Orthoptera: Gryllidae). In: Choe JC, Crespi BJ ed. The Evolution of Mating Systems in Insects and Arachnids. Cambridge, NY: Cambridge University Press, 89–109.