

Correspondences

Self-recognition in crickets via on-line processing

Alexandra Capodeanu-Nägler¹, James Rapkin², Scott K. Sakaluk³, John Hunt², and Sandra Steiger^{1,*}

Self-referent phenotype matching, the ability of animals to use aspects of their own phenotype as a referent in discrimination decisions, is believed to play a significant role in nepotistic interactions and mate choice in a wide range of taxa [1]. An individual's ability to assess the similarity between its own phenotype and that of the individuals it encounters can provide a reliable measure of relatedness, thereby facilitating inbreeding avoidance, optimal outbreeding or altruistic

behavior towards kin [2,3]. Although self-referencing is believed to be widespread, definitive evidence is scarce and its role in recognition controversial, in part, because of the difficulty in ruling out early exposure to close kin and the possibility that individuals imprint on maternal cues early in their ontogeny, either during birth or via cues encountered upon hatching from eggs [1,4]. An equally important, yet unanswered question is whether individuals that perform self-referencing imprint on their own traits at an early stage, relying on this memorized template in subsequent interactions, or whether no memory is formed but individuals use their own phenotype directly in comparison with other individuals (i.e., 'on-line processing') [3]. Finally, animals may possess 'recognition alleles', in which both the phenotypic cues and the knowledge of the cues have a genetic basis [5]. Here we show in the decorated cricket, *Gryllodes sigillatus*, that female mate choice can be manipulated by

experimentally altering a female's own olfactory cues. We found exclusive evidence both for the existence of a chemosensory self-referencing mechanism and that females do not rely on an innate or early learned template but rather directly use their own phenotypic cues in comparison.

Recent studies of *G. sigillatus* have demonstrated a female preference for novel males over previous mates [6,7]. As in the majority of insects, the outermost layer of the cuticle of these crickets is composed of cuticular hydrocarbons (CHC) that serve both as an evaporation barrier and in chemical communication. Female CHC profiles exhibit significant additive genetic variation, providing each female with her own unique olfactory cues [7]. During copulation, CHCs are physically transferred from females to males, making males' profiles more similar to that of females [7]. In mate-choice trials, focal females showed a significant aversion to males perfumed with

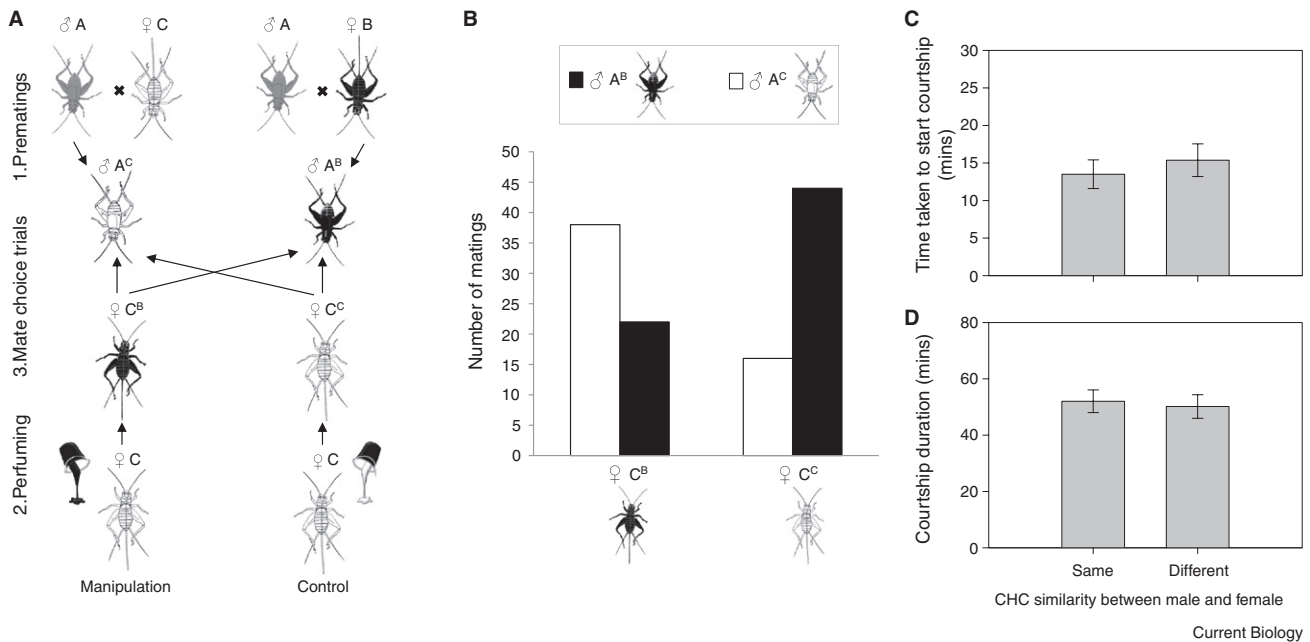


Figure 1. Chemosensory self-referencing in the decorated cricket.

(A) Schematic diagram of the experimental procedure. Males from inbred line A were randomly assigned to mate either with a female of line B or line C (focal females) for a natural transfer of CHCs from females to males. Due to the natural CHC transfer, male CHC profiles become more similar to that of the females, depicted in the figure as a change in color [7]. Although females also acquired some CHCs from males during copulation [7], this is not shown in the figure, as all focal females received these CHCs from the same male line during mating. Twenty-four hours later, focal females of line C were experimentally perfumed with the CHCs of females of line B (manipulation; n = 60) or of their own line (control; n = 60). Subsequently, they were given a choice between their previous mates or the males previously mated to females of line B. Letters in superscript indicate the line from which the CHC profile was acquired. There was no difference in (C) time taken to start courtship (P = 0.95) and (D) courtship duration (P = 0.51) of males bearing similar or dissimilar CHCs to the focal female (see Supplemental Information).

the CHC extracts of their inbred female siblings [7]. These findings strongly suggest that female crickets rely on a self-referent mechanism, thereby using their own CHC cues to discriminate against previous mates. However, it is also possible that females imprint on maternal cues early on in their ontogeny, and rely on these learnt associations in future comparisons; insect eggshell layers are derived from maternal secretions [8], and embryos undoubtedly encounter maternally derived chemical cues upon hatching. One potentially powerful method to demonstrate self-referencing is to change an individual's own phenotype, which — provided that the self-template is not genetically hardwired or only established during an early phase — should induce predictable types of recognition errors.

Here, we made use of inbred lines, which exhibit very low variation in CHC profiles of individuals within lines, but high variation between lines [7] (see Supplemental Information, published with this article online). We manipulated the CHC profile of females of one inbred line (line C) by applying CHCs of females from another (line B) on their cuticle (see Supplemental Information). In a control group, we perfumed females from one line (line C) with the CHCs of females from their own inbred line (Figure 1A). After a short recovery period, female mating preferences were examined. Females from both treatments were given a choice between two males of line A, a familiar one with which they had mated the night before (i.e., before manipulation of their own CHC profile) and an unfamiliar one that was previously mated to a female from line B (Figure 1A). If females rely on a self-referencing that compares their own current olfactory phenotype with that of a prospective mate, we would expect that chemically manipulated females would re-choose their previous mating partner and avoid mating with the male bearing a similar profile to their own. In contrast, control females should avoid mating with their previous mating partner [7]. To rule out the possibility that males actively contribute to the outcome of mate choice trials, we compared courtship effort of males encountering females

bearing similar or dissimilar CHC profiles. For all matings in our experiment we measured, firstly, the time taken for a male to initiate courtship after first contact with the female and, secondly, the interval between the male first producing a courtship call and when females chose to mate with one of them (i.e., courtship duration).

In accordance with the self-referent hypothesis, females of the two treatments showed a difference in mate preference (contingency table analysis, $\chi^2_1 = 16.29$, $p < 0.0001$; Figure 1B). Females chose the male matching their own current olfactory phenotype, thereby preferring the male bearing dissimilar cues to their own (i.e., the manipulated females preferred their previous mating partner, whereas the control females showed a preference for the novel male). There was no difference in courtship effort between males bearing the same or different CHCs to their mating partner, and so differential courtship cannot explain these results (Figures 1C,D and see Supplemental Information).

Although we cannot rule out the possibility that some form of memorization is involved in this recognition process, and that the self-template is updated over very short intervals, the most parsimonious interpretation is that no higher integration centers of the insect brain are involved, but rather female crickets use 'on-line processing' to directly compare their own phenotype to that of an encountered male. Analogous to the suggestion of Ozaki *et al.* [9] for nest-mate recognition in ants, we hypothesize that the female crickets' olfactory receptor neurons are desensitized to their own scent due to sensory adaptation, and that this affects their perception of mates bearing similar or dissimilar odor cues. This explanation also corresponds to theoretical models proposed for nestmate recognition in social insects [10]. As chemical signatures can change over time, sensory adaptation is more flexible than a template stored in long-term memory.

In conclusion, our study provides the first unequivocal evidence for the existence of a chemosensory self-referencing mechanism that is based on on-line processing. Although this mechanism is largely unstudied,

we believe that it likely plays an important role in promoting polyandry in insects and may represent a widespread mechanism allowing individuals to find their genetically most compatible partner.

Supplemental Information

Supplemental Information contains experimental procedures, one figure, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.10.050>.

Acknowledgments

A.C. was supported by the ERASMUS program KOOR/BEST. J.H. was supported by NERC, BBSRC and a Royal Society Fellowship. S.K.S. was supported by NSF and S.S. by a DFG grant (STE 1874/3-1).

References

1. Hauber, M.E., and Sherman, P.W. (2001). Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* 24, 609–616.
2. Petrie, M., Krupa, A., and Burke, T. (1999). Peacocks lek with relatives even in the absence of social and environmental cues. *Nature* 401, 155–157.
3. Hain, T.J.A., and Neff, B.D. (2006). Promiscuity drives self-referent kin recognition. *Curr. Biol.* 16, 1807–1811.
4. Hare, J.F., Sealy, S.G., Underwood, T.J., Ellison, K.S., and Stewart, R.L. (2003). Evidence of self-referent phenotype matching revisited: airing out the armpit effect. *Anim. Cogn.* 6, 65–68.
5. Blaustein, A.R. (1983). Kin recognition mechanisms: phenotypic matching or recognition alleles? *Am. Nat.* 121, 749–754.
6. Ivy, T.M., Weddle, C.B., and Sakaluk, S.K. (2005). Females use self-referent cues to avoid mating with previous mates. *Proc. R. Soc. B* 272, 2475–2478.
7. Weddle, C.B., Steiger, S., Hamaker, C.G., Ower, G.D., Mitchell, C., Sakaluk, S.K., and Hunt, J. (2013). Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets: a potentially universal mechanism facilitating polyandry in insects. *Ecol. Lett.* 16, 346–353.
8. Zeh, D.W., Zeh, J.A., and Smith, R.L. (1989). Ovipositors, amnions and eggshell architecture in the diversification of terrestrial arthropods. *Q. Rev. Biol.* 64, 147–168.
9. Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T., and Yamaoka, R. (2005). Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* 309, 311–314.
10. van Zweden, J., and d'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons. In *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*, G.J. Blomquist and A.-G. Bagnères, eds. (Cambridge, UK: Cambridge University Press), pp. 222–243.

¹Institute of Experimental Ecology, University of Ulm, 89081 Ulm, Germany.

²College of Life and Environmental Sciences, Centre for Ecology and Conservation, University of Exeter, Tremough Campus, Penryn, TR10 9EZ, UK. ³Behavior, Ecology, Evolution and Systematics Section, School of Biological Sciences, Illinois State University, Normal, IL 61790-4120, USA.

*E-mail: sandra.steiger@uni-ulm.de