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A statistical approach to identify nestmate recognition cues

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The ability of social insects to discriminate nestmates from non-nestmates is mainly achieved through chemical communication. To ultimately understand this recognition and its decision rules, identification of the recognition cues is very useful, if not essential. Although cuticular hydrocarbons are the prime suspect, identifying the exact recognition cues for specific species has remained a daunting task, partly due to the sheer number of odour variables. Perhaps unsurprisingly, one of the few species where the recognition cues have been identified, *Formica exsecta*, has only around ten hydrocarbons on its cuticle. In this study we use previous results on this species to search for likely nestmate recognition cues in two other species of ants, *Camponotus aethiops* and *Monomorium pharaonis*. Employing chemical distances and observed aggression between colonies, we first ask which type of data normalization, centroid, and distance calculation is most diagnostic to discriminate between nestmate recognition cues and other compounds. We find that using a 'global centroid' instead of a 'colony centroid' significantly improves the analysis. One reason may be that this approach, unlike previous ones, provides a biologically meaningful way to quantify the chemical distances between nestmates, allowing for within-colony variation in recognition cues. Next, we ask which cluster of hydrocarbons most likely represents the cues that the ants use for nestmate recognition, which shows less clear results for *C. aethiops* and *M. pharaonis* than for *F. exsecta*, possibly due to the number of compounds in their respective profiles. Nonetheless, some compound groups behave better than others, showing that this approach can be used to identify candidate compounds to be tested in bio-assays, and eventually crack the sophisticated code that governs nestmate recognition.