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Original Article

Kin discrimination increases with odor distance in the German cockroach

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Kin recognition mediates altruistic behavior and inbreeding avoidance in many animal societies. So far, evidence for accurate kin recognition, when individuals distinguish fine scale differences in genetic relatedness, in social insects is mixed. Although this ability should be counter selected to reduce risks of nepotism in eusocial colonies, accurate kin recognition may be beneficial in less integrated societies where genetic conflicts are reduced. Here we show that gregarious cockroaches *Blattella germanica* discriminate multiple levels of relatedness and identify inherited cuticular odors as potential kin recognition cues. When given a choice between aggregation sites containing either full siblings or less related conspecifics, cockroaches showed an increasing preference for resting with full siblings with increasing genetic distance between stimuli groups, from 50% of choices in the presence of half siblings or cousins, to 60.7% with less related cockroaches from the same strain, and 72.9% with cockroaches from a different strain. Examination of the cuticular hydrocarbon profiles of 288 nymphs and their 54 parents revealed that the chemical distance between cockroaches was negatively correlated to their relatedness. Using a Bayesian animal model approach for quantitative genetic analyses, we identified several highly heritable methyl-branched alkanes as good candidates for kin recognition cues. Our results suggest that kin recognition is based on genetically inherited odors in this gregarious insect and highlight mechanistic similarities with nestmate recognition in eusocial species.

Key words: **aggregation, animal model, *Blattella germanica*, cuticular hydrocarbons, kin recognition, odor–gene covariance.**

INTRODUCTION

Kin recognition, the ability to discriminate kin from nonkin, is taxonomically widespread, from microorganisms (Ostrowski et al. 2008) to humans (Krupp et al. 2012), and can be mediated by various mechanisms (Hepper 1991). In social species, kin recognition enables individuals to direct altruistic behavior (Hamilton 1987) and/or avoid inbreeding (Pusey and Wolf, 1996). Insects are no exception (Holman et al. 2013c). Surprisingly, however, since Greenberg's (1979) pioneering work on the primitively eusocial sweat bee *Lasioglossum zephyrum*, showing that guards selectively block the entry of conspecifics to the nest based on fine scale levels of relatedness, accurate kin recognition has remained difficult to demonstrate in insect societies (Boomsma and d'Ettorre 2013; Breed 2014).

In the advanced eusocial species, such as termites, ants, some bees, and wasps, kin recognition typically occurs at the level of colony membership through variations of cuticular odors (see reviews by Lenoir et al. 1999; van Zweden and d'Ettorre 2010). More accurate (within-colony) recognition seems to be absent or at least hard to detect (Tarpy et al. 2004; Boomsma and d'Ettorre 2013; but see Arnold et al. 1996; Nehring et al. 2011; Helanterä et al. 2013; Leadbeater et al. 2014). The prevailing hypothesis is that accurate kin recognition is selected against to reduce costly conflicts over reproduction and resource allocation that would arise from nepotistic behavior favoring more related nestmates (Keller 1997; Boomsma et al. 2003; Ratnieks et al. 2006). In these tightly integrated insect societies, the mixing of odor cues among colony members generates a uniformly distributed colony odor that provides a mechanism to counter the accuracy of within-colony recognition. In ants, for instance, chronic transfer of genetically determined cuticular hydrocarbons (CHCs) through social interactions (allogrooming and trophallaxis) (Ross et al. 1987; van Zweden et al. 2010), physical contacts with nest materials (Breed et al. 1995), or shared food (Liang and Silverman 2000) maintains a unique colony odor enabling individuals to discriminate nestmates from non-nestmates with great precision, while reducing

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the possibility to discriminate individuals of different matrilines or patrilines (Lenoir et al. 1999; van Zweden and d'Ettorre 2010; Johnson et al. 2011).

Although most research on insect kin recognition has focused on eusocial Hymenoptera, it has been proposed that accurate kin recognition based on relatedness should be more common in socially simpler (noneusocial) species, with no division of labor, lowlevels of cooperation, and therefore lower costs of nepotism (Fellowes 1998; Costa 2006; Lihoreau et al. 2012; Wong et al. 2014). In such groups, fine scale kin recognition may favor altruistic behavior and allow for inbreeding avoidance via disassortative mating.

Gregarious insects, such as the German cockroach (*Blattella germanica*), are good models for exploring this hypothesis. These domiciliary cockroaches live in metapopulations in which individuals can freely circulate (Rust et al. 1995). Adults and nymphs from multiple families (matrilineages) form large aggregations when resting during the day and feeding at night, thereby creating considerable potential for individuals of different kin classes to interact (Lihoreau et al. 2012). The probability that cockroaches from geographically distant populations encounter is also very high, due to passive dispersion by human activities in their urban habitat (Rust et al. 1995). Recent studies indicate that *B. germanica* cockroaches can discriminate their full siblings from nonsiblings of unknown relatedness, enabling them to avoid inbreeding when choosing mating partners (Lihoreau et al. 2007, 2008; Lihoreau and Rivault 2010) or to form kin groups when choosing resting sites (Rivault and Cloarec 1998; Lihoreau and Rivault 2009). By aggregating with kin, cockroaches may gain inclusive fitness benefits through the improved physiological and behavioral development of their close relatives, resulting from enhanced temperature and humidity conditions inside the groups known as "group effects" (see review by Lihoreau et al. 2012). However, it remains unknown whether cockroaches can discriminate conspecifics more accurately based on their degree of relatedness. Like in the eusocial Hymenoptera, *B. germanica* cockroaches discriminate kin and nonkin based on quantitative variations in their CHC profiles but the absence of allogrooming and trophallaxy considerably reduces odor mixing between individuals, suggesting that recognition cues show a direct association between the genotype and the phenotype (Lihoreau and Rivault 2009). We therefore hypothesized that the observed binary discrimination between full siblings and nonsiblings is the expression of a more accurate kin recognition system enabling cockroaches to discriminate fine scale differences in genetic similarity.

To test this hypothesis, we examined the ability of *B. germanica* cockroaches to discriminate classes of conspecifics with increasing genetic distance in binary choice tests for resting partners. We analyzed the relationship between the genetic and chemical distances among individuals to test if these could be used for accurate kin recognition. Finally, we applied a Bayesian animal model approach to estimate heritabilities of CHCs and identify candidate kin recognition cues.

METHODS

Study organisms

All experiments were conducted in 2009. We used *B. germanica* cockroaches of 2 laboratory strains originating from wild individuals caught in 2 French cities in 2008. Strain A (our reference strain) was established from ca. 100 adults collected in Rennes (48°06′43″N, 1°40′27″W). Strain B was established with ca. 100 adults collected more than 500 km away in Dijon (47°19′00″N, 5°01′00″E). Both strains were maintained well isolated from each other in large rearing cages (*l*: 80 cm, *w*: 30 cm, *h*: 102 cm) at 25 °C, 60% humidity, and under a 12:12 h light:dark cycle (light on at 8:00 AM) during approximately 4 generations (10 months). Cockroaches were provided with water, turkey food pellets, and cardboard shelters *ad libitum*. Adults were allowed to mate freely within the rearing cages.

To create lines of known pedigree relatedness, we collected mature oothecae from randomly sampled females in both laboratory strains. Each ootheca was isolated in a plastic rearing box (*h*: 80 mm, diameter (Ø): 50 mm) with a damp cotton wool until hatching of the F1 generation (Figure 1). Because *B. germanica*

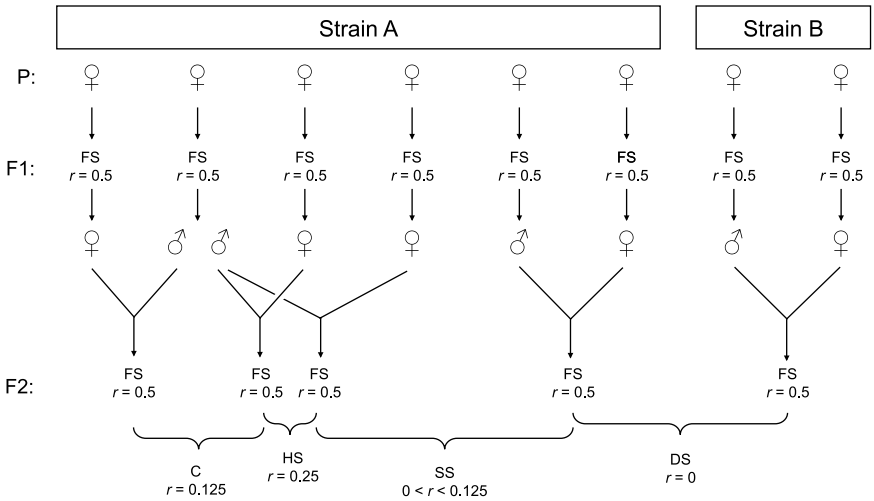

Figure 1
Rearing protocol to obtain cockroach lines of known pedigree relatedness (*r*) from 2 strains (A and B). Mature oothecae were collected from parent females (P) sampled in the strains. The F1 generation of full-sibling (FS) nymphs were reared until adulthood. F1 males and females were paired to produce the F2 generation of nymphs, in which 5 kin classes were identified: full siblings (FS: nymphs from the same parents), paternal half siblings (HS: nymphs from the same father but different, unrelated mothers), first cousins (C: nymphs from full-sibling fathers and unrelated mothers), same strain members (SS: nymphs from unrelated parents from strain A), and different strain members (DS: nymphs from different strains). Third instar nymphs from the F2 generation were used in behavioral and chemical analyses.

females mate only once, nymphs from the same ootheca were full siblings (Lihoreau and Rivault 2010). Shortly after reaching adulthood, males and females of the F1 generation were paired according to their relatedness (r) to produce the F2 generation (Figure 1). Five kin classes of F2 nymphs were then identified: full siblings (FS: $r = 0.5$), paternal half siblings (HS: $r = 0.25$), first cousins (C: $r = 0.125$), unrelated same strain members (SS: $0 < r < 0.125$), and unrelated different strain members (DS: $r = 0$). Third instar nymphs (~30 days after eclosion from the oothecae) of the F2 generation were used for choice tests and chemical analyses. Adults of the F1 generation were also kept for chemical analyses. Each cockroach was used only once.

Choice tests

We worked with nymphs because they exhibit the strongest aggregation behavior (Lihoreau et al. 2012). Focal nymphs were given a simultaneous choice between 2 resting sites, each containing a stimulus kin group of 15 third instar nymphs (Figure 2a). Tests were performed in a circular arena (large Petri dish, h: 15 mm, Ø: 140 mm), in which the 2 resting sites (small Petri dishes, h: 15 mm, Ø: 30 mm) containing the stimuli groups were placed upright 100 mm apart (see example in Supplementary Figure S1). The resting sites were closed with a plastic mesh (Ø: 1 mm) so that the focal nymph could not enter but only have antennal contacts with the stimulus groups through the mesh. Two hours before the beginning of the tests, cockroaches of the F2 generation were anaesthetized under light CO_2 in order to select and prepare the experimental individuals. From this pool of individuals, randomly selected focal nymphs were isolated in 5-ml Eppendorf tubes and groups of stimulus nymphs were placed in the resting sites.

A test consisted in releasing a focal nymph by opening the Eppendorf tube in the middle of the arena at the beginning of the light phase of the photoperiod (10:00 AM) and recording its position 24 h later. Cockroaches thus experienced a long resting phase (light on from 10:00 AM to 8:00 PM), followed by a long foraging phase (light off from 8:00 PM to 8:00 AM) and a short resting phase (light on from 8:00 AM to 10:00 AM). The test was successful if the focal nymph was in physical contact with 1 of the 2 resting sites at the end of the second resting phase (see example in Supplementary Figure S1; Rivault and Cloarec 1998). In the rare cases when trials were unsuccessful, the focal nymph was generally moving in the arena and therefore not resting.

We conducted 4 combinations of binary choice tests (Figure 2a) in which focal nymphs were observed in the presence of either 1) FS and HS ($N = 212$ trials), 2) FS and C ($N = 196$ trials), 3) FS and SS ($N = 124$ trials), or 4) FS and DS ($N = 152$ trials) (the numbers of successful trials are given in the results). For each combination of tests, we pseudorandomized the relative positions (left/right) of the stimuli groups in the arena to avoid potential biases due to side preferences by cockroaches. Neither cockroaches nor resting sites were marked so that data collection was strictly blind. Matching of behavioral data with relatedness data was made later for the statistical analyses.

All the analyses were conducted in R v.3.2.1 (R Development Core Team 2015). To compare the proportions of choices made for FS across the 4 combinations, we ran a general linear mixed model (GLMM) with binomial error and logit link, using the function "glmer" (R package *lme4*; Bates et al. 2015). In the model, choice was included as explanatory variable. Because the same stimuli groups were used in multiple trials, we included ootheca identity for

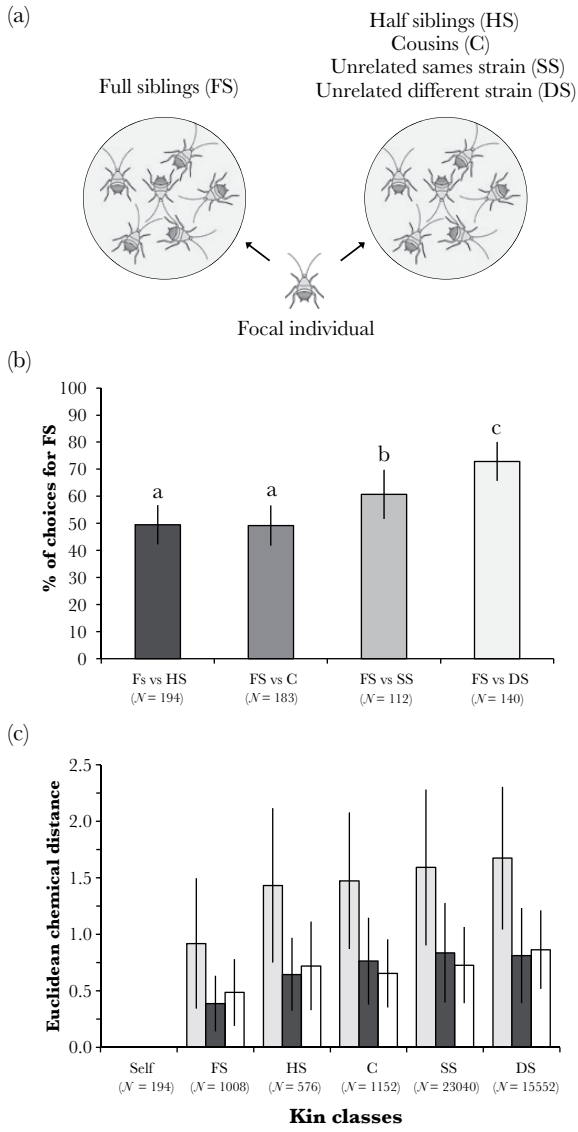

Figure 2

(a) Scheme of the choice test. Cockroaches were given a choice between a resting site containing full siblings (FS) and another containing less related conspecifics (half siblings (HS), first cousins (C), unrelated same strain members (SS), or unrelated different strain members (DS); see details in Figure 1). (b) Results of the choice test. Proportion of choices for FS are given with 95% confidence intervals. N is the number of successful trials, that is, when either of the 2 sites was chosen. The proportion of choices for FS increased with the genetic distance between stimuli groups irrespective of the matrilines of each group and of the side (left/right) in which the FS group was presented (GLMM with binomial error, $F_{3,622} = 5.98$, $P < 0.001$). Different letters above bars indicate significant pairwise differences (z-test: $P < 0.05$). (c) Relationship between kin distance and chemical distance. The Euclidean chemical distance between CHC profiles (light gray: all 25 compounds; dark gray: 5 most heritable compounds; white: 5 least heritable compounds) was calculated for each pair of cockroaches and then averaged according to the 5 kin classes. In all cases, there was a significant negative relationship between chemical distance and pedigree relatedness (Mantel test; all 25 compounds: $z = 3174.23$, $P < 0.001$; 5 most heritable compounds: $z = 1604.82$, $P < 0.001$; 5 least heritable compounds: $z = 1492.51$, $P < 0.001$). Mean and standard deviation are given. The distance to self is, by definition, 0. N is the number of pairs that were averaged in each kin class. The relatedness of SS was set to 0.0625, but results were similar when set to either 0 or 0.125.

each of the 2 groups as random factors. We also included the side of the FS group (left/right) as a random factor. For each combination of choice, we assessed whether cockroaches showed a significant preference for 1 of the 2 stimuli groups using a two-tailed sign test (binomial test with an equal probability 0.5 of choosing either group).

Chemical analyses

We analyzed the cuticular odor profiles of 288 nymphs and 54 adults. From 36 oothecae (27 oothecae for strain A and 9 oothecae for strain B), we extracted the profiles of 8 nymphs per ootheca (FS group) and their 2 parents. In strain A, this resulted in 216 nymph profiles (8 nymphs × 27 oothecae) and 45 parent profiles (27 females and 18 males, because some FS groups had the same father). In strain B, the extraction of 3 female and 6 male parents failed, resulting in 72 nymph profiles (8 nymphs × 9 oothecae) and 9 parent profiles (6 females and 3 males). All the nymphs used for the chemical analyses originated from the same oothecae as those used in the behavioral choice tests, so that the same kin relationships could be measured (Figure 1).

The extractions were done by immersing frozen cockroaches individually in 1.5 ml of dichloromethane (99%) for 2 min (Lihoreau and Rivault 2009). Extracts were dried in a nitrogen stream and re-diluted in 20 µl of dichloromethane before injection of a 1-µl sample in a gas chromatograph (Agilent 6850, Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a CP Sil5-CB column (30 m × 0.320 mm internal Ø). The temperature protocol started at 90 °C (held for 3 min), increased to 230 °C at 70 °C/min, then to 320 °C at 5 °C/min (held for 10 min). The carrier gas was helium. We identified the CHC peaks from the chromatograms obtained in the software Galaxie v1.7 (Agilent Technologies) using reference retention times (Rivault et al. 1998; Lihoreau and Rivault 2009). The 25 CHC peaks specific to *B. germanica* were present in all profiles and included in the analyses (see complete list in Figure 3). Raw peak areas were transformed to relative abundances by log-ratio normalization (Aitchison 1986) before any further analyses. Analyses with scaled peak areas (mean-centered and scaled to unit variance) gave essentially the same results (Supplementary Tables S3 and S4). All data used for the chemical analyses are available in Supplementary Texts S1–S5.

Heritability estimates of CHCs

To assess the degree to which CHC profiles were heritable, we first tested whether pairwise chemical distances of entire profiles were negatively correlated with pairwise pedigree relatedness. The Euclidean distance between all possible pairs of individuals was calculated using the pairwise differences in relative abundance of all 25 CHC compounds. Using Mantel tests with 1000 permutations (to correct for the pseudoreplication of using pairs; R package *ape*; Paradis et al. 2004), this chemical distance matrix was compared with a pedigree relatedness matrix for each pair. The relatedness of SS relationships was set to 0.0625 (see Figure 1). Setting this value

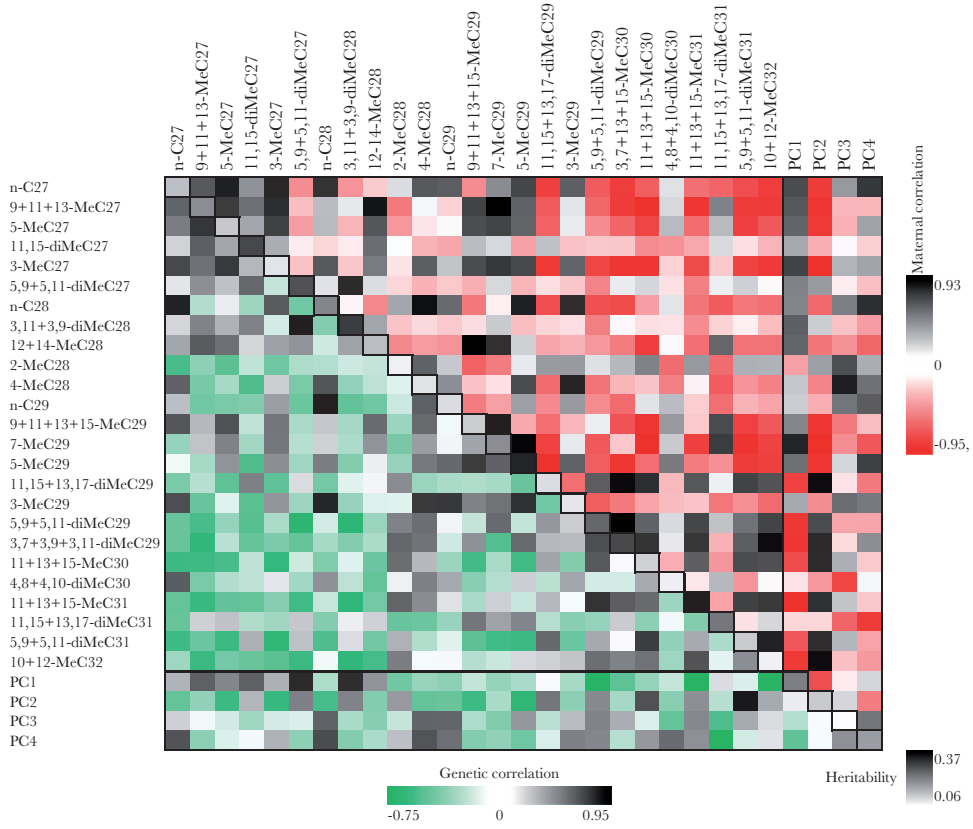

Figure 3
Heritability estimates (diagonal), genetic correlations (lower triangle), and maternal correlations (upper triangle) of CHCs using the animal model. Estimates were obtained from Bayesian mixed-effect models for each combination of 2 variables, with developmental stage (nymph/adult) as fixed variable and strain (A/B) and maternal environment (ootheca) as random variables. Heritability estimates were averaged over all models in which the compound occurred (see details in main text). PCs were extracted from an unscaled PCA including all individuals and all compounds. All numerical values are available in Supplementary Table S1.

to either 0.000 or 0.125 gave effectively the same results. We then repeated these calculations using only the 5 compounds with the highest heritability estimates (see below and Figure 2c).

To assess the heritability (h^2) of compounds, and thus which compounds are most informative for kin recognition, we analyzed the CHC data using the animal model approach (Lynch and Walsh 1998; Wilson et al. 2010). This Bayesian approach uses mixed-effect models to decompose phenotypic variance into genetic and environmental variance. Animal models allow for the estimation of the heritability of a trait and correlations between traits (e.g., due to pleiotropy, linkage disequilibrium, or experiencing the same maternal environment). We used the R package *MCMCglmm* (Hadfield 2010) to run Bayesian bivariate models for each pairwise combination of CHC variables and the first 4 principal components (PCs) of a principal component analysis (PCA). The developmental stage of the individual (nymph or adult) was included as a fixed factor, whereas strain (A or B) and maternal environment (FS groups from the same ootheca) were random factors. Models included the known pedigree of grandmothers (F0), mothers and fathers (F1), and offspring (F2), and the distribution of CHC variables was set to Gaussian (Supplementary Text S4). Each model ran for 55 000 iterations, sampling every 50th data point, and had a burn-in of 5000 iterations, thus resulting in 1000 permutations. The heritability estimate of each compound was taken as the average estimate of all 30 bivariate models containing that compound. Heritability estimates obtained by the animal model were compared with estimate values obtained with alternative approaches of paternal half sibling covariance analyses and parent–offspring regressions (Supplementary Text S6 and Tables S3 and S4). The R code used for estimating heritabilities is available in Supplementary Text S7.

We calculated genetic correlation and maternal correlations between each combination of variables. Genetic correlations may be due to for example pleiotropy or linkage disequilibrium, whereas maternal correlations between traits may be due to sharing the same ootheca nymph environment. For 3 of the compounds (n-C_{27}, n-C_{29}, and 10+12-MeC_{32}), these correlation coefficients with all other compounds were regressed against the chain length of the carbon backbone of the respective compound, using standard linear models in R.

RESULTS

Choice tests

Cockroaches given a choice between a resting site containing FS and a resting site containing less related conspecifics (HS, C, SS, or DS; Figure 2a) made a choice for 1 site in 92% of the 684 trials. This proportion of successful tests was similar across the 4 choice combinations (FS vs. HS: 91.5% of 212 trials, FS vs. C: 93.4% of 196 trials, FS vs. SS: 90.3% of 124 trials, FS vs. DS: 92.1% of 152 trials; Chi-square test, $\chi^2_3 = 1.04$, $P = 0.792$). Cockroaches did not discriminate closely related FS and HS or FS and C. Of the 194 nymphs in the FS–HS trials, 96 associated with FS and 98 with HS (sign test, $P = 0.943$; Figure 2b). Of the 183 nymphs in the FS–C trials, 90 associated with FS and 93 with C (sign test, $P = 0.883$; Figure 2b). By contrast, cockroaches discriminated FS and less related conspecifics from the same strain or from a different strain. Of the 112 nymphs in the FS–SS trials, 68 associated with FS and 44 with SS (sign test, $P = 0.$ 0.029; Figure 2b). Of the 102 nymphs in the FS–DS trials, 102 associated with FS and 38 with DS (sign test, $P < 0.001$; Figure 2b). Thus overall, cockroaches showed an increasing preference for FS as the genetic distance between the 2 stimuli groups increased, from about 50% of individuals choosing FS in the presence of HS (49.5%) and C (49.2%), to 60.7% in the presence of SS and 72.9% in the presence of DS. These choices were independent of the matrilines of the focal nymph, of the matrilines of the stimuli groups, and of the side (left/right) in which stimuli groups were presented in the arena (GLMM with binomial error, $F_{3,622} = 5.98$, $P < 0.001$).

Heritability estimates of CHCs

Chemical distances over all 25 CHC compounds increased with lower relatedness between individuals (Mantel test, $\mathcal{Z} = 3174.23$, $P < 0.001$; Figure 2c), indicating that the CHC profile as a whole is significantly genetically determined. Similar results were obtained using only the 5 most and the 5 least heritable compounds (mentioned below) ($\mathcal{Z} = 1604.82$, $P < 0.001$ and $\mathcal{Z} = 1492.51$, $P < 0.001$, respectively; Figure 2c). Therefore, there is enough heritable chemical information in the CHC profiles to allow cockroaches to discriminate the 5 kin classes.

The heritability analysis based on animal models revealed that several methyl-branched alkanes are the most heritable compounds and therefore may be most informative cues for kin recognition (Figure 3; see numerical version in Supplementary Table S1). The compounds 11,15-diMeC_{27}, 3,11+3,9-diMeC_{27}, 5-MeC_{29}, 3,7+3,9+3,11-diMeC_{29}, and 11+13+15-MeC_{31} showed the highest heritabilities, with h^2 values ranging from 0.278 to 0.372. In contrast, the compounds 3-MeC_{27}, 2-MeC_{28}, 3-MeC_{29}, 4,8+4,10-diMeC_{30}, and 10+12-MeC_{32} showed the lowest heritabilities, with h^2 values ranging between 0.065 and 0.076. Together, the first 4 PCs of the PCA explained 83.3% of the variance, but only PC1 (explaining 51.4% of the variance) showed high heritability (Figure 3). The compounds 5,9+5,11-diMeC_{27}, 3,11+3,9-diMeC_{27}, 11+13+15-MeC_{31}, and 10+12-MeC_{32} had the highest (positive or negative) loadings on this principal axis (Supplementary Table S2). This indicates that the major variation in the CHC dataset was due to these compounds. Heritability estimates obtained with paternal half sibling covariance analyses and parent–offspring regressions gave similar results (Supplementary Text S6 and Tables S3 and S4).

Patterns of pairwise genetic correlations between CHCs indicate that negative correlations are the most frequent between compounds with a short chain length and compounds with a long chain length, as exemplified in Figure 3 (red corner of lower triangle). In contrast, positive correlations are more frequent among CHCs of either short or long chain length (regression of genetic correlation coefficients on chain length; n-C_{27}, $\beta = -0.206$, $P = 0.003$; n-C_{29}, $\beta = 0.042$, $P = 0.508$; 10+12-MeC_{32}, $\beta = 0.250$, $P < 0.001$). The pattern of maternal correlations (upper triangle in Figure 3) is very similar to the genetic correlations and perhaps even clearer (regression of maternal correlation coefficients on chain length; n-C_{27}, $\beta = -0.259$, $P = 0.001$; n-C_{29}, $\beta = -0.065$, $P = 0.245$; 10+12-MeC_{32}, $\beta = 0.285$, $P < 0.001$). This similarity is likely due to the fact that FS experienced the same maternal environment (same ootheca). Interestingly, the 3 compounds with chain length 27 and high heritability (11,15-diMeC_{27}, 5,9+5,11-diMeC_{27}, and 3,11+3,9-diMeC_{27}) have very low maternal correlations with any compound and high positive genetic correlations with each other, showing that their relative abundances are not affected by environmental factors.

DISCUSSION

Using binary choice experiments, we found that *B. germanica* cockroaches express an increasing preference for aggregating with full

siblings as the genetic distance between stimuli groups increases, from no preference in the presence of close kin (half siblings or first cousins), to moderate and strong preferences with less related individuals from the same strain or a different strain. The degree of variation between the CHC profiles of cockroaches reflects their genetic relatedness and thus provides the necessary information for kin recognition. The 5 methylated alkane peaks showed high relative heritability, implicating these as good candidates for kin recognition cues.

Our results build on previous observations that cockroaches can discriminate the cuticular odors of full siblings from that of nonsiblings of unknown relatedness (Lihoreau and Rivault 2009) or odors of conspecifics of their strain from that of conspecifics of other strains (Rivault and Cloarec 1998), suggesting that discrimination of multiple kin classes is mediated by genetically determined variation of odor profiles. The fact that we could not detect behavioral discrimination between close kin in our assays raises the question of whether cockroaches cannot perceive differences between these classes of kin, or whether individuals do not respond to perceived odor differences in an aggregation context. Calculations of chemical distances suggest that there is enough information available for enabling accurate recognition of close kin. Therefore, it is likely that a different behavioral context, where the costs of discrimination errors are much higher, may have brought different results. For instance, accurate identification of full siblings may be pivotal for mate choice to enable adults with limited dispersal abilities to avoid incestuous mating and minimize the costs of inbreeding (Lihoreau et al. 2007, 2008; Lihoreau and Rivault 2010). The selective pressure for disassortative mating is thought to be an essential driver for the evolution and maintenance of polymorphic genetic odor cues (CHC profiles) underpinning kin recognition and behavioral discrimination in many animal societies (Crozier 1986; Holman et al. 2013c).

Analyses of the CHC profiles of nymphs with known pedigree relatedness and their parents indicate that several methyl-branched alkanes (notably 3,x-dimethyl alkanes) were most heritable and therefore most informative cues for kin recognition, whereas the linear alkanes showed relatively low heritability (Figure 3). In eusocial insects, alkenes and methyl-branched alkanes, rather than linear alkanes, typically mediate nestmate recognition (e.g., honey bees: Dani et al. 2005; wood ants: Martin et al. 2008; carpenter ants: van Zweden et al. 2009) and have also been found to be more heritable than the linear compounds (van Zweden et al. 2010; Holman et al. 2013b). Methyl-branched alkanes have also been reported as most informative compounds for social recognition in other arthropods, for instance in the subsocial spider *Stegodyphus lineatus* where all dimethyl and several monomethyl alkanes present on the cuticle of spiderlings have the highest discriminative power in separating families (Grinsted et al. 2011). However, compounds such as 3-MeC_{27}, 4-MeC_{28}, n-C_{29}, and 3-MeC_{29}, which are often associated with reproductive status in wasps and ants (Holman et al. 2013a; van Zweden et al. 2014; van Oystaeyen et al. 2014), show low heritability in *B. germanica*. Interestingly, some of the strongest genetic correlations are observed between these compounds irrespective of relatedness. This supports the idea that these CHCs may be more associated with the physiological state rather than the genetic background in insects, although this requires further testing.

Heritability patterns, as revealed by the animal models, also yield information about the biosynthesis process of CHCs. In particular, we notice that there are generally strong negative genetic and maternal correlations between compounds of short and long chain length. This may be ascribed to the working of the protein *elongase* in the synthesis process. This protein controls chain length specificity by elongating fatty acyl-CoA groups until the intended chain length is reached (Blomquist 2010), but uses the same precursors for similar compounds of different chain length thus causing a trade-off for synthesizing compounds with short and long chain lengths. Another remarkable pattern is that the compounds of high heritability and short chain length (11,15-diMeC_{27}, 5,9+5,11-diMeC_{27}, and 3,11+3,9-diMeC_{27}) show little correlation with other compounds, whereas those of high heritability and long chain length show strong correlations with other compounds. This suggests that these compounds are fairly independent of other compounds or maternal effects and may most accurately reflect relatedness between individuals. These results call for further investigations based on systematic correlations between all compounds of the cuticular in other insect species.

CHCs have long been identified as nonvolatile social cues used by cockroaches to forage and to find aggregation sites (Rivault and Cloarec 1998; Amé et al. 2004; Jeanson and Deneubourg 2006; Lihoreau et al. 2010). It is therefore likely that kin recognition based on CHCs shapes the long-term spatiotemporal distribution of cockroaches in natural contexts (Bell et al. 2007). Because cockroaches typically form single large aggregations instead of splitting into smaller groups, provided that the carrying capacity of the resting site (shelter) can sustain the whole population (Amé et al. 2006), accurate kin recognition could lead to a structuring of kin groups within resting aggregations. Population genetic studies have consistently reported only weak genetic structures of *B. germanica* populations across large spatial scales (between rooms, apartments, buildings, cities, or continents), presumably because of the important effect of passive dispersion mediated by humans (Cloarec et al. 1999; Crissman et al. 2010; Booth et al. 2011; Vargo et al. 2014). However, none of these studies have examined fine scale genetic structures within single aggregations and how they may change through time.

Whether kin aggregation by cockroaches is an adaptive response or a by-product of central place foraging is still an open question. By prioritizing kin groups, nymphs may share the benefits of grouping with their closest relatives (e.g., faster development due to increased ambient temperature (Lihoreau and Rivault 2008), reduced water loss (Dambach and Goehlen 1999), increased ability to locate food (Lihoreau et al. 2010) or shelters (Canonge et al. 2011), accelerated escape behavior (Laurent Salazar et al. 2013)) and thus possibly gain inclusive fitness benefits. Alternately, kin discrimination could result from the tendency of cockroaches to always return to their familiar resting site between foraging phases (Rust et al. 1995; Laurent Salazar et al. 2015) and thus search for locations with a familiar odor. In our experiments where cockroaches were raised in family aggregations (groups of full siblings), it is possible that the focal nymphs simply favored sites containing the most similar odor to that experienced before the tests. Cross-fostering studies in which cockroaches are raised in groups of unrelated individuals will help deciphering the role of odor experience and genetic relatedness in the observed decisions.

Over the past decades, research on ants and bees has led to the consensus that fine scale kin recognition is counter selected in the advanced eusocial insects to avoid costs of nepotism (Keller 1997; Boomsma et al. 2003; Ratnieks et al. 2006; Boomsma and d'Ettorre 2013). Our study in a gregarious (noneusocial) insect shows that such kin recognition abilities based on odor–gene covariance (Todrank and Heth 2003) can evolve and be maintained in simple

societies where the costs of nepotism are low relative to the benefits of avoiding inbreeding. The idea that the accuracy of social recognition systems may be linked to the cost/benefit balance of nepotism in insect societies is consistent with the observation that kin informative cues can be selectively masked or expressed in socially flexible species, as for instance in the European Earwig (*Forficula auricularia*) where CHCs specific to patrilines are concealed in juveniles but not in adults (Wong et al. 2014). In this subsocial insect, odor masking may minimize kin biased competition and cannibalism among nymphs from different patrilines constituting the brood, whereas kin informative cues may favor inbreeding avoidance in adults (Wong et al. 2014). Evidence of accurate kin recognition in solitary insects also supports this hypothesis (e.g., crickets: Thomas and Simmons 2011).

Beyond bringing fundamental knowledge on the behavioral ecology of *B. germanica*, our results also provide novel insights for the evolution of social recognition systems in cockroaches and termites (Blattodea), a phylogenetic group that has received relatively little attention but provides considerable interest for comparative research on insect social evolution (Bell et al. 2007). The utilization of CHCs for accurate kin recognition in gregarious cockroaches raises the possibility that this mechanism could have served as physiological ground (precursor) for the establishment of nestmate recognition in more advanced subsocial cockroaches and the transition to eusociality in termites (Dronnet et al. 2006). This hypothesis is consistent with behavioral observations of the subsocial wood feeding cockroach *Cryptocercus punctulatus*, a sister group of termites (Inward et al. 2007; Djernæs et al. 2012), indicating that individuals recognize members of different families using close range olfactory cues and occasionally attack conspecifics from neighboring families (Seelinger and Seelinger 1983). Further examination of social recognition systems across Blattodea species exhibiting various levels of social complexities holds considerable promises for exploring the evolution of insect communication and sociality based on comparative research with the Hymenoptera.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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