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Kin recognition via cuticular hydrocarbons shapes cockroach social life

Mathieu Lihoreau and Colette Rivault
UMR 6552, Ethologie Animale et Humaine, CNRS—Université de Rennes 1, Campus Beaulieu, Avenue du Général Leclerc, 35042 Rennes, France

Genetic relatedness plays a key role in the organization and the functioning of societies. A large diversity of species has developed kin recognition abilities, allowing individuals to discriminate conspecifics in relation to relatedness. In social insects, many studies showed that discrimination generally acts at the level of nestmateship and only few studies report kin recognition abilities. Our results highlight the importance of kin recognition in shaping social life in the urban cockroach *Blattella germanica* (L.) and present a complete description of the recognition system from expression to action components. Cockroaches of all developmental stages (nymphs and adults) discriminate siblings from nonsiblings independently of any prior social experience. Preference is context dependent so that siblings are preferred as social partners, whereas nonsiblings are preferred as mating partners. Discrimination is based on quantitative differences of cuticular hydrocarbons that are perceived through antennal contacts. As individual cuticular profiles remain stable over time, they constitute reliable discrimination cues correlated with relatedness. Our results offer interesting perspectives for the study of kin recognition and for the understanding of evolution toward sociality in insects.
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Genetic relatedness plays a key role in the organization and functioning of social groups of many species. Hamilton (1964) predicted that individuals would benefit by behaving altruistically toward their closest kin, thus increasing their indirect fitness. Although kin selection theory provides the most powerful explanation for the evolution of kin-biased behavior in family groups (Wilson 1971; Crozier and Pamilo 1996), kin discrimination can be beneficial in other types of association, as for example, when choosing mates (Bateson 1983; Waldman 1988; Fellowes 1998).

Many vertebrate as well as invertebrate species have developed complex kin recognition abilities based on cues correlated with genetic relatedness (Beecher 1982; Fletcher 1987; Blaustein et al. 1988; Hepper 1991; Sherman et al. 1997; Holmes 2004). Like other recognition systems, kin recognition occurs during an encounter between a cue bearer and an evaluator and can be described in terms of expression, perception, and action components (see Starks 2004). The expression component includes emission or acquisition of recognition cues by the cue bearer (e.g., olfactory, sound, and/or visual cues). The perception component concerns the evaluator's cue-sensing and processing mechanisms (e.g., comparison of cues to a template). The action component focuses on the physiological, developmental, or behavioral response by the evaluator (e.g., acceptance/rejection). Appropriate responses depend both on the encounter context and on the level of match between cues and templates.

Several putative models, still debated, aim to explain the underlying mechanisms of kin recognition (see Mateo 2004). A prevailing categorization lists 4 major mechanisms including spatial location, prior association or familiarity, phenotype matching, and recognition alleles, sensu Hamilton (1964). However, a new theoretical framework, elaborated mainly from interpretations of recent empirical evidence in vertebrates, considers only the mechanisms allowing relatedness assessment sensu stricto, irrespective of spatial location and familiarity (Barnard 1990; Grafen 1990; Tang-Martinez 2001; Todrank and Heth 2003). Kin recognition could thus occur either through learning a neural template from self that is compared with the cues of the encountered individual ("armpit effect" Dawkins 1982; "self-referent phenotype matching" Holmes and Sherman 1982, 1983) or by selective peripheral structures precluding the need for a higher perceptual matching process (e.g., Osaki et al. 2005). The lack of persuasive empirical evidence gives little credit to the allele recognition model (for discussion, see Tang-Martinez 2001).

Surprisingly, since Greenberg's famous report on kin recognition in sweat bees (Greenberg 1979), only few studies have evidenced kin recognition abilities in insects. Social recognition systems that have been described in detail in eusocial Hymenoptera (ants, bees, and wasps) and Isoptera (termites) generally act at the level of nestmateship (Carlin and Hölldobler 1983; Isingrini et al. 1985; Breed and Julian 1992; Robinson et al. 1999; Gamboa 2004; Osaki et al. 2005). They coincide with kin recognition in the rare cases when colonies are headed by a single queen, who has mated only once, and without queen turnover (Lenoir et al. 1999). In these species, discrimination is generally mediated by differences in individual cuticular hydrocarbon (CHC) profiles (Singer 1998; Vander Meer et al. 1998; Lahav et al. 1999; Lenoir et al. 2001; Howard and Blomquist 2005). Most reports on kin recognition in insects concern solitary species (Herre 1985; Simmons 1989; Ueno and Tanaka 1996; Lizé et al. 2006). Very few reports concern social species (sensu Costa and Fitzgerald 2005), and their kin recognition systems are still poorly documented (Hemiptera: Kasuya 2000; Loeb et al. 2000; Coleoptera: Agarwala and Dixon 1993; Joseph et al. 1999; Pervez et al. 2005). Knowing that individuals of social species associate with conspecifics for a wide array of activities, including foraging, resting, or mating, kin recognition should drive choice of preferred partners in relation to associated costs and benefits in many contexts (Fellowes 1998). Analyses of these recognition systems would improve our understanding of the organization and the functioning of these societies (Costa 2006).

Address correspondence to M. Lihoreau. E-mail: mathieu.lihoreau@univ-rennes1.fr.
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Here, we investigated kin recognition abilities of the urban cockroach, *Blattella germanica* (L.). This is a group-living species where individuals of all generations (6 nymphal instars and adults) share a common shelter, exploit a common foraging area, and usually remain in their hatching area (Rivault 1989, 1990). Females mate only once in their lifetime and produce successive batches of full-sibling nymphs (sex ratio 1:1, $r = 0.5$). Consequently, group members share high levels of relatedness within an aggregate and among neighboring aggregates (Cloarec et al. 1999). These cockroaches use strain odors and CHCs to select resting sites and to form aggregates (Rivault and Cloarec 1998; Jeanson et al. 2005; Amé et al. 2006). In addition, when choosing a mate, adults avoid close inbreeding and its subsequent impairment of direct fitness (Lihoreau et al. 2007). Based on all these converging data, we hypothesized that preferences observed when choosing an aggregation site or a sexual partner are based on kin recognition abilities. Like in many insects, CHCs could be involved in discrimination processes.

The aim of this study was to investigate the role of kin recognition in shaping social interactions among *B. germanica* cockroaches and to describe the recognition system in detail, from expression to action components. First, we investigated the occurrence of kin discrimination in 2 different contexts, both in nymphs and in adults, focusing on the choice of 1) social and 2) mating partners. Then, we investigated underlying mechanisms by testing the efficiency of CHC extracts to induce partner choice in these 2 contexts. Behavioral analyses were complemented by chemical analyses that evaluated interindividual differences of CHC profiles in relation to relatedness.

MATERIALS AND METHODS

Experimental individuals

Experimental subjects came from our *B. germanica* (L.) laboratory stock culture. Insects were reared and tested at 25 ± 1 °C under a 12:12 h light:night photocycle. They were provided water, turkey food pellets, and shelters ad libitum. Experimental individuals (nymphs and adults) were reared in groups of siblings according to the following procedure until they were tested. Mature oothecae were collected from gravid females (freely mated with males in our stock culture) and placed individually in plastic rearing boxes (50 mm high × 80 mm in diameter). After hatching, nymphs were reared in groups in these boxes without being manipulated, except nymphs tested in experiment 1d that were isolated when teneral (before cuticular tanning) and reared individually in similar boxes. All the individuals from the same ootheca were siblings ($r = 0.5$) and those from 2 different oothecae were nonsiblings ($0 \leq r < 0.5$); r is an estimation of the coefficient of relatedness (Wright 1922). Each experimental individual was tested only once.

Choice of social partners by nymphs

To investigate the influence of relatedness on choice of social partners by nymphs, one nymph was given a choice between 2 resting sites containing either groups of conspecifics or odors of conspecifics.

Test subjects were second-instar nymphs. They were tested in plastic Petri dishes (15 mm high × 140 mm in diameter) containing 2 potential resting sites. Resting sites were 2 small cylinders (15 mm long × 30 mm in diameter) placed on their side, 10 cm apart. Test nymphs could not enter these cylinders, only rest on them. When the cylinders contained groups of conspecifics (15 second-instar nymphs), one end of the cylinder was either closed with a double plastic wire mesh (mesh = 0.5 mm) that prevented test nymphs from having antennal contacts with the enclosed nymphs or closed with a single plastic wire mesh (mesh = 1 mm) that allowed antennal contacts. When cylinders were empty, one cylinder end was covered with a filter paper disc (15 mm in diameter) scented with conspecific CHC extracts. CHC extracts were obtained by dipping 30 second-instar nymphs in 1.5 mL of dichloromethane for 2 min (Rivault et al. 1998). These extracts were evaporated under nitrogen flow, collected in 10 µL of dichloromethane, and applied onto a filter paper disc fixed onto one end of the cylinder. Test nymphs could contact the CHC extracts freely with their antennae.

Tests were set up during the light phase of the photocycle because *B. germanica* is nocturnal and rests in large aggregates during the diurnal part of the photocycle. Data were collected when nymphs had spent an entire photocycle in the test dishes and had had the opportunity to make a fair choice between the 2 resting sites after a complete activity cycle (Rivault and Cloarec 1998; Amé et al. 2006). During tests, nymphs were deprived of water and food. Tests were considered successful only when nymphs were resting on one of the cylinders. The proportions of tests when nymphs rested on each type of resting site were calculated.

Five experiments investigated the influence of relatedness on choice of social partners by nymphs (Table 1):

Experiment 1a: Test nymphs were given a choice between 2 cylinders closed with double wire mesh (setup preventing antennal contacts). One cylinder was empty and the other contained conspecific (nonsibling) nymphs.

Experiment 1b: Test nymphs were given a choice between 2 cylinders closed with double wire mesh (setup preventing antennal contacts). One cylinder contained sibling nymphs and the other nonsibling nymphs.

Experiment 1c: Test nymphs were given a choice between 2 cylinders closed with a single wire mesh (setup allowing antennal contacts). One cylinder contained sibling nymphs and the other nonsibling nymphs.

Experiment 1d: Test nymphs that had been reared in isolation since hatching (separated when teneral) were tested under the same experimental conditions as in experiment 1c (setup allowing antennal contacts).

Experiment 1e: Test nymphs were given a choice between 2 cylinders covered, one with CHC extracts of sibling nymphs and the other with CHC extracts of nonsibling nymphs (setup allowing antennal contacts).

Choice of social and mating partners by adults

We investigated the influence of relatedness on the choice of partners by adults by giving 1 test male a choice in a Y-olfactometer either between 2 potential partners placed in retention or between the odors of 2 potential partners. Tests evaluated either social partner preference or mating partner preference of males.

The glass Y-olfactometer was composed of a starting stem (100 mm long and 10 mm internal diameter) and 2 arms (100 mm long and 10 mm internal diameter). A pump (New-Air, Loreggia, Italy) pushed charcoal-purified humidified air at a constant flow rate (180 mL/min), controlled by a flowmeter (Brook, Hatfield, PA), through the 2 arms of the olfactometer.

When test males were given a choice between 2 partners, each potential partner was placed in retention in a small plastic tube (10 mm long and 3 mm in diameter). One end of the tube was left open so that the head and antennae of the cockroach emerged. Consequently, test males could exchange antennal contacts with cockroaches in retention. One cockroach in retention was placed at the end of each olfactometer arm.

Table 1
Choice of social partners by nymphs

Experiment	Rearing	AC	*N* tot.	*N* uns.	S1	S2	*N*1	*N*2	*P*
1a	Grouped	−	32	7	EC	NS	7	25	0.043
1b	Grouped	−	148	31	NS	S	59	58	1.000
1c	Grouped	+	162	31	NS	S	67	95	0.034
1d	Isolated	+	147	22	NS	S	61	86	0.047
1e	Grouped	+	56	11	CHC NS	CHC S	15	30	0.036

Methods and results of experiments 1a–e. Exp., experiment name (1a–e); Rearing, rearing conditions (grouped or isolated); AC, antennal contact prevented (−) or allowed (+); *N* tot., total number of tests; *N* uns., number of unsuccessful tests; S1, stimuli presented at site 1 (EC, empty cylinder; NS, nonsibling nymphs); S2, stimuli presented at site 2 (S, sibling nymphs); *N*1, number of tests where nymphs chose site 1; *N*2, number of tests where nymphs chose site 2; *P*, binomial tests.

When test males were given a choice between conspecific odors, these odors were CHC extracts obtained by dipping 5 males in dichloromethane for 2 min. These extracts were evaporated under nitrogen flow, collected in 10 µL of dichloromethane, and then applied on filter papers (10 mm long and 1.5 mm large) fixed onto empty plastic tubes (10 mm long and 3 mm in diameter), and one was placed at the end of each olfactory arm.

Tests were made during the night phase, when cockroaches are active, and data were recorded under red light as it is not detected by cockroaches (Koehler et al. 1987). Experimental subjects were 6-day-old virgin males. Before a test, males were placed individually in Eppendorf tubes that were opened in front of the entrance of the Y-olfactometer so that they could walk freely into the olfactometer, thus avoiding stress due to manipulation by the experimenter or to recent CO_2 anesthesia. After entering the olfactometer, test males were observed continuously for 5 min and time spent in each arm was recorded. Tests were considered successful only when males visited both olfactometer arms. Male choice was evaluated by the arm in which they stayed the longest during a test. The proportions of tests when males chose each type of stimulus were calculated.

Choice of social partners

Two experiments investigated the influence of relatedness on the choice of social partners by adult males (Table 2):

Experiment 2a: Test males were given a choice between a sibling male and a nonsibling male.

Experiment 2b: Test males were given a choice between CHC extracts of sibling males and CHC extracts of nonsibling males.

Choice of mating partners

When sexually receptive, *B. germanica* females emit a sexual calling pheromone that attracts males (Nojima et al. 2005). Then, reciprocal antennal contacts trigger male courtship. The presence of a cockroach in retention (either a male or a female) with freely moving antennae placed in a flow of sexual pheromone mimics a sexual context and triggers male courtship. As in this context test males can collect information from only a restricted part of their partner's body, they do not seem to be able to discriminate gender at this stage. This experimental protocol evaluated male choice of mating partner. The flow of sexual pheromone was obtained by placing 100 virgin females in a large glass container (20 mm internal diameter and 80 mm long) connected, with a T-glass stopper, to each Y-olfactometer arm. Pheromone flow was thus pushed equally through the 2 arms so that it attracted males to the cockroaches in retention placed at the extremities of the olfactometer arms. Only males in retention were used in both contexts for 2 reasons: first, so that we can compare data from choice of social partner (experiment 2a) to data from choice of mating partner and second we avoid thus the influence of the varying states of receptivity of females.

Two experiments investigated the influence of relatedness on the choice of mating partners by males (Table 2):

Experiment 2c: Test males were given a choice between a sibling male and a nonsibling male under a sexual pheromone flow.

Experiment 2d: Test males were given a choice between CHC extracts of sibling males and CHC extracts of nonsibling males under a sexual pheromone flow.

Analyses of CHC profiles

Gas chromatography

CHC profiles were analyzed in a gas chromatograph (GC, Varian 3400) equipped with a flame ionization detector (FID) operating at 300 °C and a split/splitless injector at 250 °C (splitless mode). The column was a CP Sil5-CB (Varian, Palo Alto, CA) (25 m long × 0.25 mm internal diameter with a 0.25-µm-thick film). The carrier gas was helium. The temperature program started at 90 °C for 3 min and then increased gradually first to 230 °C at 15 °C/min and then to 320 °C at 5 °C/min (final time 10 min). Data were collected and treated

Table 2
Choice of social partners and mating partners by males

Exp.	Context	*N* tot.	*N* uns.	S1	S2	*N*1	*N*2	P_1
2a	Social	110	2	NS	S	43	65	0.043
2b	Social	160	4	CHC NS	CHC S	65	91	0.044
2c	Sexual	80	0	NS	S	50	30	0.033
2d	Sexual	80	2	CHC NS	CHC S	50	28	0.017

Methods and results of experiments 2a–d. Exp., experiment name (2a–d); Context, social or sexual; *N* tot., total number of tests; *N* uns., number of unsuccessful tests; S1, stimuli used in arm 1 (NS, nonsibling); S2, stimuli used in arm 2 (S, sibling); *N*1, number of tests where males chose arm 1; *N*2, number of tests where males chose arm 2; P_1, binomial tests.

with Galaxie 1.7.4.5 software (Varian). Chromatogram peaks were identified by comparison with our previously published chromatograms of *B. germanica* CHCs (Rivault et al. 1998).

CHC profiles

First, we evaluated interindividual differences of CHC profiles in relation to relatedness among individuals by comparing the CHC profiles of 100 first-instar nymphs originating from different oothecae (10 siblings from each of 10 different oothecae). Nymphs were frozen and then dipped individually in 1.5 mL of dichloromethane for 2 min. Individual cuticular extracts were evaporated under nitrogen flow and collected in 10 µL of dichloromethane. Samples of 1 µL were analyzed by GC.

Second, we evaluated intraindividual stability of CHC profiles in relation to social environment (group composition) and in relation to time (in days) by comparing CHC profiles of 1-day-old and 15-day-old nymphs. Ten groups of 5 sibling and 10 groups of 5 nonsibling newly hatched first-instar nymphs were reared in plastic boxes (50 mm high × 80 mm in diameter) until they were 15 days old (second-instar nymphs). CHCs were collected with a solid-phase microextraction fiber (SPME, 100 µm polydimethylsiloxane, Supelco, Bellafonte, PA). The cuticle of each nymph was rubbed all over with a SPME fiber for 10 s, once on day 1 and again later on day 15. This nondestructive sampling method provided CHC profiles of the same individuals at different times. SPME fibers were desorbed for 15 min in GC.

Statistical analyses

Data were analyzed using R 2.2.1 software (Ihaka and Gentleman 1996). χ^2 Homogeneity tests compared frequencies of unsuccessful behavioral tests between experiments, and binomial tests analyzed binary choice test data (experiments 1 and 2).

GC peak areas of each cockroach cuticular profile were transformed into percent areas, prior to statistical treatment. A discriminant analysis (DA) evaluated the variability of CHC profiles of 100 nymphs originating from 10 different oothecae in relation to relatedness among individuals and investigated whether the 10 predefined groups of nymphs, that is, oothecae, could be discriminated on the basis of their chemical profiles. Wilk's lambda and the percentage of correct assignments of individuals to their respective groups evaluated the quality of the DA. Partial Wilk's lambda were calculated to evaluate the contribution of each peak to the discriminative power of the global model. Squared Mahalanobis distances, which measure distances between clouds of points, were calculated between groups. To avoid limitations inherent to the analysis of compositional data, prior to DA, each peak area was transformed according to Aitchinson's formula (Aitchinson 1986):

$$Z_{ij} = \mathrm{Ln}\left[\frac{Y_{ij}}{g(Y_j)}\right],$$

where Z_{ij} is the transformed area of peak i for individual j, and $g(Y_j)$ is the geometric mean of the areas of all peaks for individual j (Steiger et al. 2007). All the 25 compounds of CHC profiles previously identified (Rivault et al. 1998) were included in the DA. Indices of similarity between CHC profiles of individuals were calculated to investigate the stability of CHC profiles in relation to group composition and to time using Nei's formula (Nei 1972):

$$I_{xy} = \frac{\sum^n X_i \times Y_i}{\sqrt{\sum^n X_i^2 \sum^n Y_i^2}},$$

where I_{xy} is the similarity index between CHC profiles of individuals x and y, n is the number of peaks, X_i is the area (%) of peak i for individual x, and Y_i is the area (%) of peak i for individual y (De Biseau et al. 2004). Nei indices were calculated for each pair of individuals within groups using the 25 compounds included in the DA. The mean Nei indices were then calculated for each group of nymphs, both for day 1 and for day 15. A 2-way repeated measures analysis of variance (ANOVA) evaluated the effect of group composition (siblings/nonsiblings) and the effect of time (day 1/day 15) on mean Nei indices of similarity between the CHC profiles of the 5 individuals within a group.

RESULTS

Choice of social partners by nymphs

Experiments 1a–e evaluated the influence of relatedness on choice of social partners by nymphs when selecting a resting site (Table 1, Figure 1). As the proportions of unsuccessful tests did not differ significantly among the 5 experiments (experiments 1a–e, range: 13.02–21.88%, $\chi^2 = 6.643$, $P = 0.156$), they were all discarded from further statistical analyses.

When given a choice between an empty cylinder and a cylinder containing conspecific nymphs (setup preventing antennal contacts), test nymphs chose significantly more often the site containing conspecifics (experiment 1a). As the double wire mesh on the cylinders prevented antennal contacts, test nymphs had necessarily detected the presence of conspecifics from a distance. When given a choice between 2 cylinders closed with double wire mesh (setup preventing antennal contacts), one containing siblings and the other nonsiblings, test nymphs showed no significant preference for one of the sites

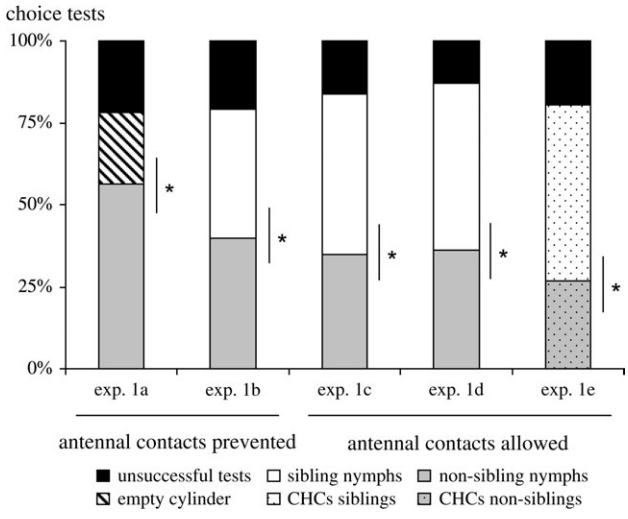

Figure 1
Choice of social partners by nymphs. Nymphs were presented odor stimuli in a setup that either prevented or allowed antennal contacts. Test nymphs were given a choice between (experiment 1a) an empty cylinder and a cylinder containing conspecific nymphs (setup preventing antennal contacts); (experiment 1b) a cylinder containing sibling nymphs and a cylinder containing nonsibling nymphs (setup preventing antennal contacts); (experiment 1c) a cylinder containing sibling nymphs and a cylinder containing nonsibling nymphs (setup allowing antennal contact); (experiment 1d) similar to experiment 1c, except that test nymphs were reared in isolation since hatching; and (experiment 1e) a cylinder scented with CHCs from siblings and a cylinder scented with CHCs from nonsiblings (setup allowing antennal contact). One hundred percent stacked columns give the percentage of unsuccessful tests + the percentage of tests where individuals chose each of the 2 options. *, $P < 0.050$, binomial test.

(experiment 1b). Under the same test conditions, but when the cylinders were closed with only a single mesh (setup allowing antennal contacts), test nymphs significantly preferred the site containing siblings (experiment 1c). These results demonstrate that relatedness influences the choice of social partners by nymphs. The fact that nymphs discriminated siblings from nonsiblings only when they could have antennal contacts with them (experiment 1c) shows that kin discrimination cues are not detected from a distance but are necessarily perceived through contact. A similar experiment (setup allowing antennal contacts) indicated that nymphs reared in isolation since hatching (separated from siblings when teneral) also chose significantly more frequently the site containing siblings than that containing nonsiblings (experiment 1d). This suggests that nymphs do not have to learn the phenotypes of conspecifics through prior contacts to discriminate siblings from nonsiblings.

When given a choice between 2 cylinders scented either with CHC extracts from siblings or with CHC extracts from nonsiblings (setup allowing antennal contacts), nymphs chose significantly more frequently the site scented with sibling CHCs (experiment 1e). This reveals that CHC extracts of nymphs contain discrimination cues necessary to induce sibling/nonsibling discrimination.

Although the level of discrimination errors cannot be neglected, our results demonstrate that nymphs, be they reared in a group or in isolation, prefer siblings to nonsiblings as social partners when selecting a resting site. The presence of conspecifics is detected from a distance, but kin discrimination requires antennal contacts with CHCs.

Choice of social and mating partners by adults

Experiments 2a–d evaluated the influence of relatedness on the choice of social and mating partners by males (Table 2, Figure 2). As the proportions of unsuccessful tests did not differ significantly among the 4 experiments (experiments 2a–d, range: 0–2.5%, $\chi^2 = 2.018$, $P = 0.569$), they were all discarded from further statistical analyses.

When given a choice between a sibling male and a nonsibling male, test males chose significantly more frequently the olfactometer arm containing siblings (experiment 2a). Similarly, when given a choice between sibling CHC extracts and nonsibling CHC extracts, test males chose significantly more frequently the arm containing sibling CHC extracts (experiment 2b). This shows that males preferred siblings as social partners and that contact with CHCs is sufficient to induce discrimination. These results agree with our results for nymphs presented above (experiment 1c–e).

When given the same choice as in experiment 2a, but under a sexual pheromone flow that simulated a sexual context, males chose significantly more frequently the arm containing the nonsibling male than that containing the sibling male (experiment 2c). Similarly, when given the same choice as in experiment 2b, but under a sexual pheromone flow, test males chose more frequently the arm containing nonsibling CHC extracts than that with sibling CHC extracts (experiment 2d). Thus, in a sexual context, males were preferentially attracted by nonsibling partners.

Once again, the level of discrimination errors cannot be neglected. Nevertheless, our results demonstrate that males discriminate siblings from nonsiblings and that they modified their preference in relation to context. Males, like nymphs, prefer siblings as social partners but choose nonsiblings as sexual partners.

Analyses of CHC profiles

First, chemical analyses of CHC profiles evaluated interindividual differences in relation to relatedness. The 25 previously identified *B. germanica* CHCs (Carlson and Brenner 1988; Rivault et al. 1998) were found in all our extracts, whatever the extraction method (liquid or solid phase).

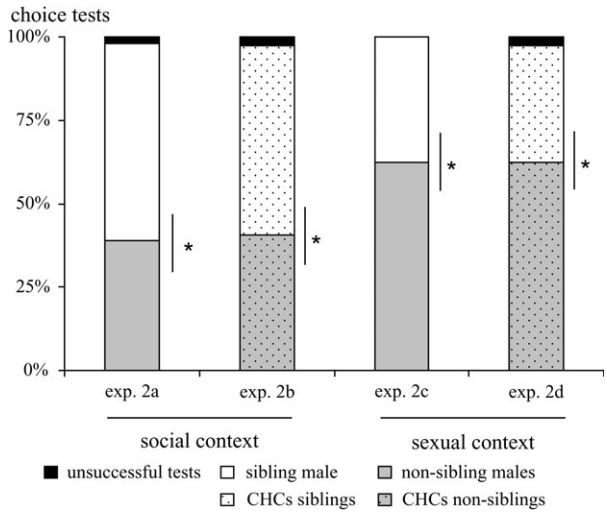

Figure 2
Choice of social partners and mating partners by males. Test males were given a choice, in a social context, between (experiment 2a) a sibling male and a nonsibling male, in the absence of sexual pheromone and (experiment 2b) CHCs from sibling males and CHCs from nonsibling males, in the absence of sexual pheromone. Test males were given a choice, in a sexual context, between (experiment 2c) a sibling male and a nonsibling male, in the presence of sexual pheromone and (experiment 2d) CHCs from sibling males and CHCs from nonsibling males, in the presence of sexual pheromone. One hundred percent stacked columns give the percentage of unsuccessful tests + the percentage of tests where males chose each of the 2 arms of the olfactometer. *, $P < 0.050$, binomial test.

A DA on the 25 CHCs clearly divided profiles of individuals according to their original oothecae (percentage of correctly assigned cases: 100%, Wilk's lambda = 0.000, $F_{207,591} = 12.542$, $P = 0.000$). Nine discriminant functions contributed significantly to discrimination among groups. Function 1 accounted for 51.12% and function 2 for 14.45% of the total variance (Figure 3). Squared Mahalanobis distances between centroids of the 10 predefined ootheca groups were statistically significant for all distances ($P < 0.001$). All except 3 of the 25 peaks (*n*-nonacosane; 12- and 14-methyloctacosane; 10- and 12-methyldotriacontane) contributed significantly to discrimination (partial Wilk's lambda < 0.80, $F > 2$, $P < 0.050$). Interindividual differences of the relative abundance of 22 peaks are thus sufficient to discriminate siblings from nonsiblings. These results reveal quantitative similarities among CHC profiles of individuals that belong to the same ootheca and quantitative differences between CHC profiles of individuals from different oothecae.

Second, we evaluated intraindividual stability in relation to group composition and time. Two-way repeated measures ANOVA of mean Nei indices of each group (calculated with the 25 CHCs in the DA) revealed that mean Nei indices were significantly influenced by group composition (groups of siblings or groups of nonsiblings) and were stable in relation to time (days 1–15) (group composition: $F_{1,18} = 4.524$, $P = 0.048$; time: $F_{1,18} = 0.055$, $P = 0.818$; group composition × time: $F_{1,18} = 0.003$, $P = 0.959$). Mean Nei indices were always significantly higher in groups of siblings than in groups of nonsiblings (honest significant differences Tukey, $P = 0.048$), confirming that the similarity of CHC profiles is higher within a group of siblings than within a group of nonsiblings.

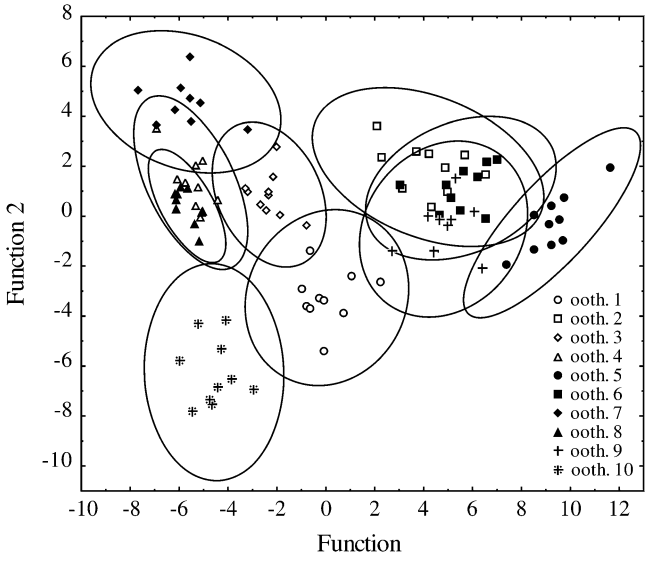

Figure 3
Interindividual variability of CHC profiles in relation to genetic relatedness. DA performed on the CHC profiles of 100 nymphs originating from 10 different oothecae (oothecae 1–10) correctly assigned 100% of the individuals in relation to their original ootheca. Scatterplot of function 1 (51.12% of variance) versus function 2 (14.45% of variance) is presented. Envelopes represent the 95% confidence ellipses.

Remarkably, Nei indices did not differ significantly between day 1 and day 15, neither for nymphs reared in a group of siblings nor for nymphs reared in a group of nonsiblings, indicating that individual CHC profiles remain stable over time. This result led us to conclude that no significant transfer of CHCs between nymphs occurs through passive cuticular contacts.

DISCUSSION

Our study highlights the key role of kin recognition in shaping social interactions in *B. germanica* and describes, for the first time, the entire recognition system, from expression to action components. We showed that 1) these cockroaches discriminate siblings/nonsiblings and their preference is context dependent, 2) discrimination is based on quantitative differences between individual CHC profiles, and 3) discrimination can occur without any previous social experience.

Preference is context dependent

These results confirm our previous findings that *B. germanica* cockroaches are able to discriminate siblings from nonsiblings (Lihoreau et al. 2007). The novelty of this study is that the discriminative response is expressed at all developmental stages and the preference depends on the context of the encounter between the cue bearer and the evaluator.

When they had a choice of social partners, both nymphs and adults preferred to associate with siblings rather than with nonsiblings. These results are consistent with the fact that nymphs prefer shelters scented with their own strain odor to shelters scented with odors of other strains (Rivault and Cloarec 1998), indicating an affinity for odors of their closest related conspecifics. *Blattella germanica* aggregations are mediated by a self-organized process driven by mutual interattraction, and thus, individuals spontaneously form a unique aggregate providing that shelter space is not limited (Jeanson et al. 2005; Amé et al. 2006). As cockroaches in interspecific aggregates form specific subgroups (Boyer and Rivault 2004; Leoncini and Rivault 2005), we hypothesized that, under natural conditions, siblings would form subgroups within large aggregates composed of individuals from diverse origins rather than segregate. Grouping is known to provide different kinds of direct fitness benefits such as lower predation risks or lower energetic costs by decreasing temperature loss or water evaporation (Krause and Ruxton 2002). When these benefits are shared by closely related individuals, group living then potentially increases indirect fitness of group members (Hamilton 1964). Tactile stimulation by *B. germanica* conspecifics significantly accelerates developmental rates of nymphs and adults (Izutsu et al. 1970; Holbrook et al. 2000; Lihoreau and Rivault 2008). Associations among siblings could thus be a strategy to increase their inclusive fitness by favoring development and survival of closely related individuals. Conversely, in a sexual context, *B. germanica* males were preferentially attracted to nonsibling partners. As adult dispersion is not the rule in this species (Rivault 1990), kin discrimination during mate choice constitutes the main strategy to avoid extreme inbreeding and subsequent deleterious effects on direct fitness (Lihoreau et al. 2007). The behavioral response of *B. germanica* cockroaches is thus context dependent and seems to optimize the evaluator's inclusive fitness.

Discrimination is based on CHC profiles

Whatever the encounter context, both nymphs and adults discriminated cuticular extracts of siblings from those of nonsiblings. As cuticular extraction in dichloromethane provides a pure fraction of the 25 identified CHCs present in *B. germanica* nymphs and adults (Carlson and Brenner 1988; Rivault et al. 1998), our behavioral results demonstrate that individual CHCs provide sufficient information for kin discrimination.

Our chemical analyses highlighted interindividual differences of the relative abundance of CHC compounds. The fact that quantitative differences of CHCs were lower among siblings, that is, intra-ootheca variability, than among nonsiblings, that is, inter-oothecae variability, indicates that differences are linked to genetic relatedness among individuals. As the significant differences concern 22 of the 25 compounds, kin discrimination by *B. germanica* is more likely to be based on differences among many compounds than among only a few. Variations of many compounds generate a large number of combinations, and consequently, the CHC patterns of 2 individuals rarely overlap completely. This is a particularly reliable mechanism to evaluate relatedness level in large aggregates that include individuals from many oothecae.

Our analyses of individual profiles revealed that they were stable in relation to social environment and remained stable over time. The CHC profile of a single nymph after its second nymphal molt (at day 15) was the same as before molting. This suggests that individuals synthesize the same CHC profile after a molt. In addition, the profiles of individuals in a group (of siblings or of nonsiblings) did not homogenize, although individuals had been in close contact for a long time. Contrary to colony members of many eusocial species that mix their cuticular profiles to form a unique colony odor (Crozier and Dix 1979; Crozier 1987; Dapporto et al. 2004), *B. germanica* cockroaches do not form a group odor. Each individual keeps its genetically inherited signature that constitutes a reliable kin discrimination signal. All our results leave the door open for odor–gene covariance that would allow graded preferences along a genetic relatedness continuum (Todrank and Heth 2003).

Discrimination is independent of social experience

Nymphs reared in isolation since hatching, isolated before their cuticle was tanned by hydrocarbons, discriminated

siblings from nonsiblings in the same proportions as cockroaches reared in groups (experiment 1c reared in a group: 45.50%; experiment 1d reared in isolation: 45.36%). The fact that naive individuals that had never had any contacts with conspecifics had the same discrimination abilities as grouped nymphs indicates clearly that relatedness assessment is not based on matching cues of an encountered individual with a reference template learned through social experience. This conclusion is supported by the fact that individuals reared with nonsiblings are still able to discriminate unfamiliar siblings from familiar nonsiblings as adults (Lihoreau et al. 2007). Consequently, *B. germanica*'s recognition abilities do not pertain to recognition of familiar individuals (e.g., recognition by prior association or by familiarity) but can be considered as kin recognition involving genetic relatedness assessment (see Todrank and Heth 2003). Although these results do not allow us to identify the precise mechanism involved in *B. germanica* kin recognition, they suggest a mechanism based either on learning one's self phenotype as a recognition template, that is, armpit effect or self-referent phenotype matching (e.g., Heth et al. 1998; Mateo and Johnston 2000) or on the absence of learning via a peripheral recognition mechanism that does not require feedback to the brain (e.g., Osaki et al. 2005). Whatever the exact mechanism, the fact that discrimination is based on consistent differences among individual CHC profiles makes it reliable, particularly in large *B. germanica* aggregates where familiar nonsiblings of all developmental stages live in close contact and interact frequently.

Conclusions

Blattella germanica has evolved sophisticated kin recognition abilities that play a key role in the choice of social and sexual partners. To our knowledge, this is the first social insect (sensu Costa and Fitzgerald 2005) for which kin recognition has been studied in its entirety, from expression to action components. Our results offer interesting perspectives for the study of recognition systems and the evolution toward sociality within insects.

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