

DIET-RELATED MODIFICATION OF CUTICULAR
HYDROCARBON PROFILES OF THE
ARGENTINE ANT, *Linepithema humile*, DIMINISHES
INTERCOLONY AGGRESSION

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Abstract—Territorial boundaries between conspecific social insect colonies are maintained through a highly developed nestmate recognition system modulated by heritable and, in some instances, nonheritable cues. Argentine ants, *Linepithema humile*, use both genetic and environmentally derived cues to discriminate nestmates from nonnestmates. We explored the possibility that intraspecific aggression in the Argentine ant might diminish when colonies shared a common diet. After segregating recently field-collected colony pairs into high or moderate aggression categories, we examined the effect of one of three diets: two hydrocarbon-rich insect prey, *Blattella germanica* and *Supella longipalpa*, and an artificial (insect-free) diet, on the magnitude of aggression loss. Aggression diminished between colony pairs that were initially moderately aggressive. However, initially highly aggressive colony pairs maintained high levels of injurious aggression throughout the study, independent of diet type. Each diet altered the cuticular hydrocarbon profile by contributing unique, diet-specific cues. We suggest that acquisition of common exogenous nestmate recognition cues from shared food sources may diminish aggression and promote fusion in neighboring colonies of the Argentine ant.

Key Words—Argentine ant, cuticular hydrocarbons, diet, invasive ants, nestmate recognition, unicoloniality.

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INTRODUCTION

Social insects have evolved a highly developed recognition system that forms the basis of social structure and communication. The signals used in nestmate recognition are primarily under genetic control; however, exogenous cues derived from nest materials (Gamboa et al., 1986; Stuart, 1987) or diet (Jutsum et al., 1979; Obin and Vander Meer, 1988; Le Moli et al., 1992; Liang and Silverman, 2000) may also play a role. Cuticular hydrocarbons have long been considered important mediators of nestmate recognition in ants (Vander Meer and Morel, 1998), with recent evidence supporting a direct role (Lahav et al., 1999; Liang and Silverman, 2000). However, the relative contribution of heritable and environmentally derived cues, including hydrocarbons, to the recognition profile is not known. Irrespective of the source, workers must learn colony-specific cues and must be able to properly evaluate cues present on newly encountered workers. Recognition cues are generally dynamic and may change throughout the life of the colony (Vander Meer et al., 1989) and exhibit seasonal variation (Ichinose, 1991). Therefore a worker must continually update its perception of colony odor in response to endogenous and external changes.

The Argentine ant, *Linepithema humile*, is one of several invasive ants in which the relative loss of territorial behavior is thought to contribute to invasion success. Introduced *L. humile* populations are unicolonial and frequently very large, and they often dominate native ant species (Suarez et al., 1999). Nestmate recognition in the Argentine ant is influenced by genetic (Tsutsui et al., 2000, 2003; Suarez et al., 2002) and environmental (Chen and Nonacs, 2000; Liang and Silverman, 2000) inputs. Holway et al. (1998) and Suarez et al. (2002) reported that aggression persisted between *L. humile* colonies despite maintenance under uniform rearing conditions, whereas Chen and Nonacs (2000) observed a decrease in *L. humile* intercolony aggression following 2 mo of laboratory rearing. Whereas Tsutsui et al. (2000) demonstrated a significant inverse relationship between *L. humile* genetic similarity and intercolony aggression, the colony pairs used by Holway et al. (1998), Chen and Nonacs (2000), and Suarez et al. (2002) were not subjected to genetic analysis. Therefore the observed changes (or lack thereof) in aggression may have resulted from different degrees of genetic similarity, with aggression between the most dissimilar pairs unlikely to change despite similar rearing conditions.

Whereas it has been suggested that loss of genetic diversity is primarily responsible for the unicolonial population structure observed in introduced Argentine ant populations (Tsutsui et al., 2000), the role of shared environmental cues such as diet in promoting unicoloniality is unknown. Shared dietary components, specifically hydrocarbons, for colonies displaying low intercolony genetic differentiation, may mask subtle inherent between-colony distinctions, thereby promoting fusion of adjacent colonies. Liang and Silverman (2000) and

Silverman and Liang (2001) demonstrated the potential of prey hydrocarbons to alter nestmate recognition in the Argentine ant, with worker exposure to specific prey eliciting aggression from colony mates. Of the many different prey exposed to *L. humile* workers, contact with the brown-banded cockroach, *Supella longipalpa*, induced the highest level of intracolony aggression (Liang et al., 2001). *S. longipalpa* has several hydrocarbons that are identical or similar to those of *L. humile*, which may be important in *L. humile* nestmate recognition (Liang et al., 2001). We propose that the opposite process can occur, whereby key *S. longipalpa*-acquired recognition chemicals attenuate differences between *L. humile* colonies, thus diminishing intercolony aggression. We compare intercolony aggression levels before and after continuous exposure to diets including *S. longipalpa*, *Blattella germanica*, or artificial diet and also measure changes in key prey-specific hydrocarbons on *L. humile* cuticle. We hypothesize that diets with *S. longipalpa* will diminish aggression the most. By documenting changes in intercolony aggression following exposure to sources of exogenous recognition cues, we hope to develop a deeper understanding of the dynamic nature of Argentine ant nestmate discrimination and its potential role in structuring populations in this invasive insect.

METHODS AND MATERIALS

Collection and Rearing of Laboratory Colonies. We used 11 colonies of Argentine ants (*L. humile*) from 11 sites in the southeastern USA—North Carolina (six): Chapel Hill (chh), Emerald Isle (emi), Greenville (gnc), Jacksonville (jac), Shallotte (sch), and Winston-Salem (for); South Carolina (two): Greenville (hto) and Greer (gwm); and Georgia (three): Barnesville (bch), Fayetteville (fay), and Griffin (grf). Ants were collected from a variety of habitats, including landscaped residential lots, natural wooded areas, or sand dunes. For each location, we established three large colonies consisting of 5000–10,000 workers, a few hundred queens, and numerous brood. Colonies were maintained in soil-free, Fluon-coated trays. Nests were plastic dishes filled with moist grooved plaster. Colonies were reared on one of three diets, each of which included a 25% sucrose solution *ad libitum* and hard-boiled eggs once a week: artificial noninsect diet (Bhatkar and Whitcomb, 1970), *S. longipalpa* male and female adults, or *B. germanica* male and female adults. All colonies were maintained at $24 \pm 1^\circ\text{C}$, $50 \pm 10\%$ RH, and a 12:12 hr light/dark cycle.

Aggression Tests (Nestmate Recognition Bioassay). We assessed the initial level of aggression between 18 colony pairs (listed below) with an assay that measured the level of aggression in single worker introductions into a foreign colony. This behavioral assay has low variance among replicates within the same colony pairing (Roulston et al., 2003). Individual intruder workers were

collected on a toothpick and introduced into rearing trays (52 × 38 cm) containing a resident colony (~10,000 workers). The responses of resident workers toward the intruder were recorded, and aggression was scored using the 0–4 scale of Suarez et al. (1999). The intruder was discarded after each trial, and subsequent trials were conducted when the residents were no longer visibly agitated (5–10 min). Ten replicates per colony pair were performed: five replicates with colony 1 as the resident and five replicates with colony 1 as the intruder. The observer who recorded the aggression level did not know the identity of the interacting colonies and was unfamiliar with the hypothesis being tested. All assays to estimate the initial aggression levels were performed within a week of collection and extraction of ants from the original nesting substrate. Data were analyzed as the maximum score per trial (Roulston et al., 2003).

Our preliminary observations indicated a possible relationship between the initial level of aggression displayed by a colony pair and that colony pair losing aggression over time, with pairs having high initial aggression maintaining it over time and colonies with moderate levels of initial aggression becoming nonaggressive. We define moderate aggression as an average score of 3.0 or lower and high aggression as a score of 3.0 or higher on a 0–4 scoring scale (Suarez et al., 1999). This assignment is based on aggression above level 3 being injurious (biting, stinging), whereas aggression below level 3 is non-injurious (mutual antennation, avoidance). Eight colony pairs were moderately aggressive: gnc–fay, for–emi, chh–bch, for–gnc, chh–grf, chh–hto, gwm–sch, and gwm–fay, and 10 colony pairs were highly aggressive: jac–fay, jac–sch, jac–chh, emi–bch, jac–hto, emi–grf, emi–chh, emi–hto, emi–sch, and jac–bch. Aggression assays and hydrocarbon analyses were repeated 140 d later for all three dietary regimes to assess changes in nestmate recognition patterns and to determine whether behavioral changes were consistent with hydrocarbon patterns. Aggression assays were performed again at day 224 to determine whether aggression had further declined with prolonged laboratory rearing.

Extraction, Isolation, and Chemical Analysis of Cuticular Hydrocarbons. Ants were killed by freezing (–20°C) prior to hydrocarbon extraction. External lipids were extracted from the cuticle by immersing 10 whole thawed ants in 1-ml hexane for 10 min, followed by a brief second rinse. The samples were gently shaken for the first and last 20 sec of the soak period. Hexane extracts were concentrated under nitrogen to ~100 µl and applied to prewetted (hexane) Pasteur pipette minicolumns filled with 500 mg of silica gel (63–200 mesh size, Selecto Scientific, GA, USA). The hydrocarbon fraction was eluted with 6-ml hexane and blown to dryness under nitrogen. The extract was redissolved in 5-µl hexane, and 1 µl was analyzed (two ant equivalents). Gas chromatography (GC) was carried out using an HP 5890 gas chromatograph equipped with a DB-1 column (30 m × 0.25 mm × 0.25 µm film thickness) and interfaced with a G1045A Chemstation (version A05.01). Oven temperature was held at 40°C

for 2 min, then increased to 200°C at 20°C/min, and then to 310°C at 40°C/min. The injector and flame-ionization detector were at 270 and 320°C, respectively. Helium was the carrier gas, and the make-up gas was nitrogen. Quantitative data were obtained by integrating the peaks and calculating the percent area under each peak. Specific peak identity was determined with hydrocarbon standards and by matching diagnostic peaks with those from prior studies (Jurenka et al., 1989; Liang et al., 2001).

Statistical Analyses. The significance of main effects (diet and initial aggression category) and their interaction was tested by using a mixed model ANOVA (PROC MIXED) in SAS 8.1 (SAS Institute, 2002). Upon finding that the effect of diet was not the same in the two aggression categories, we tested for the effect of diet on aggression loss within each of the two aggression categories with colony pairing and diet treated as random and fixed variables, respectively (ANOVA, PROC MIXED). Differences between the three dietary treatments within and across aggression categories were determined with least-squares means. To analyze the magnitude of aggression loss, we used absolute, rather than relative, aggression loss values.

We used linear discriminant analysis (LDA) (Statgraphics Plus, v. 5.1) to examine hydrocarbon divergence patterns between field-collected colonies (Initial) and the same colonies raised on each of the three diets (*Blattella*, *Supella*, and Artificial). The analysis was performed using standardized variables, and an LDA matrix was constructed with 11 colonies, belonging to each of four treatments (Initial, *Blattella*, *Supella*, and Artificial), using 27 peak percentages of the most abundant cuticular hydrocarbons. Significance tests comparing diets used the MANOVA procedure (PROC GLM). The degree of dispersion around the centroids (i.e., the degree of differentiation between colonies within a treatment) was calculated by averaging standard deviations for each of the 11 colonies across all 27 hydrocarbons within each treatment. To test whether Argentine ants acquired key prey-specific hydrocarbons, we first identified key diagnostic hydrocarbons provided by each prey. For *B. germanica*, we selected peaks corresponding to 11-, 13-, and 15-methylnonacosane and 3-methylnonacosane. Both hydrocarbons are relatively abundant in adult *B. germanica*, comprising approximately 14.5 and 10.3% of the total hydrocarbons, respectively (Jurenka et al., 1989). Furthermore, our preliminary analysis indicated that both hydrocarbons were readily acquired by Argentine ants. For *S. longipalpa*, we selected 15,19-dimethylheptatriacontane present in *S. longipalpa* at 19.0% and acquired by Argentine ants from *S. longipalpa* prey (Liang and Silverman, 2000; Liang et al., 2001). To compare changes in individual hydrocarbon levels (average level on day 140 vs. average level on day 0), we used one of two types of *t*-tests, depending on the equality of variances. A parametric *t*-test was used when the variances were homogenous. In cases where the variances were unequal, we used the Welch *t*-test with a Satterthwaite correction (Zar, 1999).

RESULTS

Analysis of the behavioral data revealed that the interaction between diet and aggression was significant (ANOVA, $F_{2,15,1} = 13.38$, $P < 0.001$). Because the diet effects were not the same in the two aggression categories, a separate analysis of diet effects for each aggression category was performed. Colony pairs experienced a significant reduction in initial aggression, irrespective of the diet (Table 1 and Figure 1). The aggression scores in colonies that were initially moderately aggressive and reared on either of the two cockroach diets decreased by ~40% ($P = 0.91$, Table 2). Ants raised on the artificial diet, however, experienced an ~70% loss in initial aggression scores, which was significantly higher than that experienced by ants raised on either *B. germanica* ($P < 0.001$) or *S. longipalpa* ($P < 0.001$). Argentine ants displaying high initial aggression experienced relatively little change in aggression, approximately 8% loss for each of the three dietary regimes. This decrease, although relatively low, was statistically significant for each of the three diets (Table 1), and the magnitude of aggression loss did not differ between dietary categories (Table 2; ANOVA, $F_{2,18} = 0.72$, $P = 0.50$). A comparison of the magnitude of aggression loss between the aggression categories revealed that moderately aggressive colony pairs lost a significantly higher proportion of their initial aggression across all dietary treatments, relative to colony pairs showing high initial aggression (Table 2). Results of aggression tests performed 84 d after the first testing revealed no further aggression loss in any of the aggression/diet categories ($P > 0.05$).

To provide another measure of the magnitude of aggression loss in both aggression categories, we recorded changes in the proportion of injurious/

TABLE 1. INITIAL AGGRESSION LEVELS AND AGGRESSION LOSS IN MODERATELY AND HIGHLY AGGRESSIVE COLONY PAIRINGS REARED UNDER THREE DIETARY REGIMES

Aggression category	Initial aggression level ^a	Aggression loss				
		Change				
		Diet	End	Absolute	Relative	P^b
Moderate	2.79 ± 0.08 (n = 8)	<i>Supella</i>	1.7 ± 0.2	1.1 ± 0.2	39.5 ± 7.1%	<0.001
		<i>Blattella</i>	1.8 ± 0.2	1.0 ± 0.2	36.7 ± 5.7%	<0.001
		Artificial	0.8 ± 0.2	2.0 ± 0.2	73.0 ± 7.0%	<0.001
High	4.00 ± 0.00 (n = 10)	<i>Supella</i>	3.7 ± 0.1	0.3 ± 0.1	8.3 ± 2.2%	0.002
		<i>Blattella</i>	3.8 ± 0.1	0.2 ± 0.1	5.5 ± 2.1%	0.009
		Artificial	3.8 ± 0.1	0.2 ± 0.1	5.8 ± 2.4%	0.018

^aInitial aggression levels are between pairs of field-collected colonies. Values reported are mean ± SE.

^bANOVA on absolute change (SAS Institute, 2002).

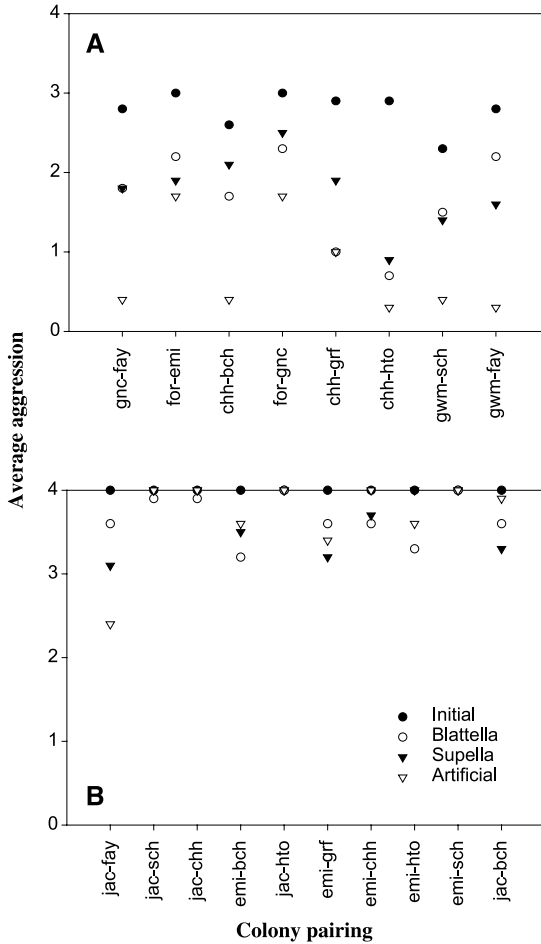


FIG. 1. Changes in intraspecific aggression in moderately (A) and highly (B) aggressive colony pairings. Field-collected colonies (Initial, closed circles) raised on one of three diets: *Blattella* (open circles), *Supella* (closed triangles), and artificial (open triangles). Mean values are reported ($n = 10$) with error bars omitted for clarity. See Methods and Materials for explanation of colony codes.

noninjurious encounters between colony pairs. Our initial aggression classification (moderate vs. high) was based on a distinction between injurious and noninjurious aggression. We classified aggression above level 3 as injurious (biting, stinging) and aggression below level 3 as noninjurious (mutual antennation, avoidance). Although the magnitude of aggression loss was statistically

TABLE 2. COMPARISON OF AGGRESSION LOSS WITHIN AND AMONG AGGRESSION CATEGORIES

Diet	Aggression	Aggression loss	Diet	Aggression	Aggression loss	P^a
<i>Supella</i>	Moderate	1.1 ± 0.2	<i>Blattella</i>	Moderate	1.0 ± 0.2	0.91
<i>Supella</i>	Moderate	1.1 ± 0.2	Artificial	Moderate	2.0 ± 0.2	<0.001
<i>Blattella</i>	Moderate	1.0 ± 0.2	Artificial	Moderate	2.0 ± 0.2	<0.001
<i>Supella</i>	High	0.3 ± 0.1	<i>Blattella</i>	High	0.2 ± 0.1	0.42
<i>Supella</i>	High	0.3 ± 0.1	Artificial	High	0.2 ± 0.1	0.25
<i>Blattella</i>	High	0.2 ± 0.1	Artificial	High	0.2 ± 0.1	0.73
<i>Supella</i>	High	0.3 ± 0.1	<i>Supella</i>	Moderate	1.1 ± 0.2	0.001
<i>Blattella</i>	High	0.2 ± 0.1	<i>Blattella</i>	Moderate	1.0 ± 0.2	<0.001
Artificial	High	0.2 ± 0.1	Artificial	Moderate	2.0 ± 0.2	<0.001

^aLeast squares analysis (SAS Institute, 2002).

significant in each of the two aggression categories, our results indicated that in contrast to pairs displaying moderate aggression, the incidence of injurious fights between highly aggressive pairs remained high. Therefore we question the biological importance of the statistically significant aggression loss between highly aggressive colony pairs. Among moderately aggressive pairs, initially 69 of 80 (86%) encounters resulted in an aggression score of 3 or 4 (three out of 80 had an aggression score of 4). At the end of the study, only 36% of *Supella*-fed ants (corresponding to a 58% reduction in the number of injurious encounters), 36% of *Blattella*-fed ants (58% reduction), and 19% of ants on the artificial diet (78% reduction) displayed level 3 aggression. In contrast, among highly aggressive colonies, initially all encounters (100/100) scored level 4. At the end of the study, 96% of *Supella*-fed ants (4% reduction), 97% of *Blattella*-fed ants (3% reduction), and 98% of ants on the artificial diet (2% reduction) still engaged in injurious aggression. Level 4 aggression remained in 71% of *Supella*-fed ants, 78% of *Blattella*-fed ants, and 79% of ants fed with artificial diet.

Argentine ants acquired significant levels of prey hydrocarbons (Figure 2)—*Blattella*: 11-, 13-, and 15-methylnonacosane ($P < 0.001$), 3-methylnonacosane ($P < 0.001$) and *Supella*: 15,19-dimethylheptatriacontane ($P < 0.001$). Because *S. longipalpa* hydrocarbons overlap the long-chain hydrocarbon profile of *L. humile* (Liang et al., 2001), field-collected colonies of Argentine ants possess relatively high initial levels of hydrocarbons specific to *S. longipalpa* (Figure 2C). However, there are certain qualitative and quantitative differences between *L. humile* hydrocarbons and those provided by *S. longipalpa*. Consequently, providing Argentine ants with *S. longipalpa* prey would likely alter the relative ratio of hydrocarbons already present as well as introduce new hydrocarbons. Furthermore, the results of discriminant analysis revealed marked

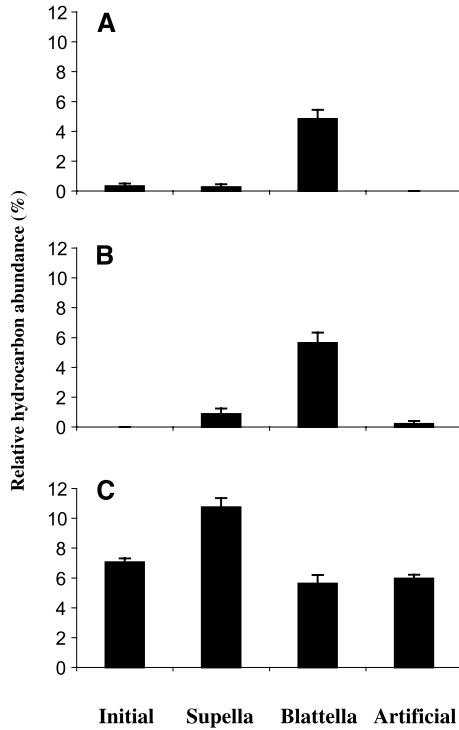


FIG. 2. Relative abundance (% total area) of key prey-derived hydrocarbons in field-collected colonies of *L. humile* (Initial) and colonies provisioned with one of prey diets: *S. longipalpa* (*Supella*) and *B. germanica* (*Blattella*). Hydrocarbons (A) and (B) are *Blattella*-derived and (C) is *Supella*-derived. (A) 11-, 13-, and 15-methylnonacosane; (B) 3-methylnonacosane; (C) 15,19-dimethylheptatriacontane. Means \pm SE for 11 colonies are presented.

divergence in cuticular hydrocarbon composition between field-collected colonies (Initial) and colonies raised on either *B. germanica*, *S. longipalpa*, or the artificial diet (Figure 3): Initial vs. *Blattella* (MANOVA, Wilk's lambda = 0.0028, $F_{26,5} = 67.04$, $P < 0.001$), Initial vs. *Supella* (Wilk's lambda = 0.010, $F_{26,5} = 18.16$, $P = 0.002$), and Initial vs. Artificial (Wilk's lambda = 0.010, $F_{26,5} = 18.94$, $P = 0.002$). The divergence between the *Blattella* and *Supella* diets was also significant (Wilk's lambda = 0.0066, $F_{26,5} = 28.88$, $P < 0.001$). Estimates of intracolony variability within treatments revealed that field-collected colonies (Initial) had the lowest variance (1.029), followed by colonies on the artificial diet (1.035), *Supella* (1.147), and *Blattella* (1.209). Changes in the proportions of the 27 most abundant cuticular hydrocarbons are summarized

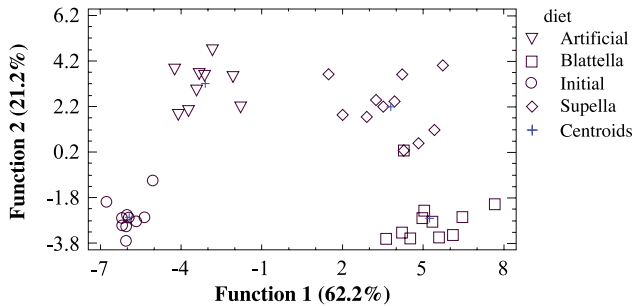


FIG. 3. Linear discriminant analysis of the 27 predictor variables (relative proportions of hydrocarbons) for 11 colonies of *L. humile* each provided a unique diet (*B. germanica*, *S. longipalpa*, and artificial).

in Table 3. Overall, colonies raised on all three diets experienced changes in the proportions of all hydrocarbons. In colonies raised on the artificial diet, six hydrocarbons significantly increased and eight hydrocarbons significantly decreased (52% of hydrocarbons changed significantly). In colonies raised on *Blattella*, five hydrocarbons significantly increased and 12 hydrocarbons significantly decreased (63% change). Colonies raised on *Supella* experienced the greatest change (67%), with seven hydrocarbons increasing and 11 hydrocarbons decreasing over the course of the study.

DISCUSSION

Our results provide further support for Argentine ant nestmate recognition being modulated, in part, by environmental factors (Chen and Nonacs, 2000; Liang and Silverman, 2000), in that rearing under controlled conditions reduces aggression between colonies. Prior studies (Liang and Silverman, 2000; Liang et al., 2001) demonstrated that exogenous hydrocarbons disrupted *L. humile* colony integrity by inducing aggression between fragments of the same colony. Herein, we determined that shared diet reduced intercolony aggression to an extent that might promote fusion of dissimilar colonies.

Changes in aggression were minimal between colony pairs that were initially highly aggressive, despite worker ants acquiring prey hydrocarbons. Therefore limited change in the pattern of intraspecific aggression within the highly aggressive pairings suggests that exogenous recognition cues did not override intrinsic cues, which may help to explain the results of other ant studies in which aggression persisted over time (Le Moli et al., 1992; Heinze et al., 1996; Holway et al., 1998; Stuart and Herbers, 2000; Suarez et al., 2002). In

TABLE 3. TWENTY-SEVEN MOST ABUNDANT CUTICULAR HYDROCARBONS AND THEIR CHANGES FOLLOWING REARING ON LABORATORY DIETS

Hydrocarbon	Diet		
	Artificial	<i>Blattella germanica</i>	<i>Supella longipalpa</i>
<i>n</i> -Heptadecane	0	0	+
Nonadecene	0	+	+*
<i>n</i> -Tricosane	—	—	—
<i>n</i> -Pentacosane	—	—	—
<i>n</i> -Heptacosane	+*	+*	+*
<i>n</i> -Octacosane	+*	+	+*
<i>n</i> -Nonacosane	+*	+*	+*
11-, 13-, and 15-Methylnonacosane	—	+*	—
3-Methylnonacosane	+	+*	+*
<i>n</i> -Triacontane	+	+*	+
<i>n</i> -Hentriacontane	+*	—	+
11-, 13-, and 15-Methylhentriacontane	—	—	—*
Dimethylhentriacontane	+*	—	+
Trimethylhentriacontane	—*	—	+
<i>n</i> -Tritriacontane	—	—*	—*
13- and 15-Methyltritriacontane	—*	—*	—*
5,15- and 5,17-Dimethyltritriacontane	—	—*	—*
5,15,19-Trimethyltritriacontane	—	—*	+*
13-, 15-, and 17-Methylpentatriacontane	—*	—*	—*
15,19-Dimethylpentatriacontane	+*	—*	—*
5,15- and 5,17-Dimethylpentatriacontane	—*	—*	—*
5,13,17- and 5,15,19-Trimethylpentatriacontane	—	—*	—*
3,13,17- and 3,15,17-Trimethylpentatriacontane	—*	—*	—
13-, 15-, 17-, and 19-Methylheptatriacontane	—*	—*	—*
15,19-Dimethylheptatriacontane	—*	—	+*
5,15- and 5,17-Dimethylheptatriacontane	—*	—*	—*
5,15,19- and 5,13,17-Trimethylheptatriacontane	—	—*	—*
Significant changes	+(6), -(8)	+(5), -(12)	+(7), -(11)

*Significant change in hydrocarbon proportion ($P < 0.05$; Student's *t*-test).

contrast, colonies in pairings that were initially moderately aggressive were strongly affected by the imposition of diet-based cues. We predicted that exposure to cockroach prey, especially *S. longipalpa*, would provide abundant hydrocarbons that could minimize between-colony recognition disparities, thereby producing the greatest decline in aggression. However, intraspecific aggression in ants fed with artificial diet diminished further than aggression in ants fed with cockroaches, indicating that the greatest change in behavior may be a consequence of the field-to-lab transition. Hydrocarbon profiles of *L. humile* raised on the artificial diet changed significantly over time; however, the hydrocarbons

(and/or chemical cues) affecting this behavioral change are unclear. Moreover, the difference in the degree of aggression loss between the two prey diets was not significant, an unexpected result given that long-chain hydrocarbons from *S. longipalpa* (33 carbons or more) added to the native *L. humile* cuticular profile induced intracolony aggression, whereas other prey items from diverse insect taxa (including *B. germanica*), having fewer or none of these long-chain hydrocarbons, induced little or no within-colony aggression (Liang et al., 2001). The action of prey hydrocarbons in promoting intercolony unity appears to differ from that inducing intracolony aggression. Perhaps the difference in the way prey hydrocarbons alter nestmate recognition in *L. humile* is related to quantitative differences in hydrocarbon levels needed to affect a behavioral change. Thus, where two antagonistic fragments were created from a single colony (Silverman and Liang, 2001), the acquisition of one or a few key hydrocarbons produced a recognizable shift in an otherwise uniform profile. In the current study, the acquisition of foreign prey hydrocarbons appeared insufficient to completely override intrinsic distinctions between colonies.

Our findings may provide insights into the evolution of unicoloniality in introduced populations of the Argentine ant. Proposed mechanisms for the transition from multi- to unicoloniality include reduced heterozygosity due to genetic drift (Tsutsui et al., 2000) and introduction to new habitats with relaxed ecological constraints (Giraud et al., 2002), with unicoloniality maintained via selection against rare recognition alleles (Tsutsui et al., 2003). Whereas Tsutsui et al. (2000, 2003) and Giraud et al. (2002) demonstrated the importance of genetic, ecological, and behavioral factors in the transition from multicoloniality to unicoloniality, we suggest that shared environmentally derived recognition cues may promote unicoloniality by masking inherent between-colony distinctions, thereby allowing adjacent colonies to fuse. Competing Argentine ant colonies will most likely interact with each other at advancing invasion fronts where they are likely to compete for nesting sites and/or food resources that provide similar nestmate recognition cues. In areas where mutually aggressive colonies exploit a common locally abundant food source, two mechanisms may work independently or together to promote fusion of neighboring colonies: diet sharing and intraspecific "dear enemy" phenomenon, whereby competing animals respond less aggressively to threats by neighbors than strangers (Temeles, 1994; Heinze et al., 1996; Langen et al., 2000). Diet sharing through cooperative use of locally abundant food sources may provide sufficient levels of critical hydrocarbons to alter nestmate recognition and promote fusion of colonies. Argentine ants, like other invasive ants, are generalist feeders (Newell and Barber, 1913; Markin, 1970), tending homoptera and scavenging living and dead arthropods. In addition, shared cues originating from nesting material may diminish intercolony aggression. The "dear enemy" phenomenon may also play a role in the Argentine ant's transition from multicoloniality to

unicoloniality. An increase in the frequency of encounters between aggressive colonies may decrease the frequency of aggression between them, especially in areas where food is abundant and intraspecific competition is limited (Foitzik and Heinze, 1998). Furthermore, repeated interactions in the field were essential for maintaining recognition among spatially isolated nests in the polydomous *Leptothorax longispinosus* with nonaggressive field colonies becoming hostile after 3 mo of laboratory rearing, thereby indicating a possible role for common environment-derived cues and/or contact between colonies in reducing aggression (Stuart and Herbers, 2000). Where Argentine ants interact and compete for resources that provide common recognition cues, cooperation among nests through a reduction in intercolony aggression may be promoted. However, an exception may occur within the native range, where *L. humile* is multicolonial despite neighboring colonies presumably having access to shared environment-derived cues. Native *L. humile* exhibit relatively high genetic diversity and high intercolony aggression (Tsutsui et al., 2000); consequently, environmentally derived recognition cues may be limited in diminishing intercolony aggression. However, colonies from invasive populations having weaker genetic structure (Tsutsui and Case, 2001; Buczkowski et al., 2004) may be more likely to fuse when exogenous cues mask colony distinctions, thereby fostering the development of unicoloniality.

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