ANIMAL BEHAVIOUR, 2002, **64**, 697–708
doi:10.1006/anbe.2002.4011, available online at http://www.idealibrary.com on IDEAL®

# Spatiotemporal patterns of intraspecific aggression in the invasive Argentine ant

**ANDREW V. SUAREZ*‡, DAVID A. HOLWAY*, DANGSHENG LIANG†, NEIL D. TSUTSUI*‡ & TED J. CASE***
*Division of Biological Sciences, Section of Ecology, Behavior & Evolution, University of California San Diego
†Clorox Technical Center, Pleasanton, California, U.S.A.
‡Center for Population Biology, Section of Ecology & Evolution, University of California Davis

*(Received 29 August 2001; initial acceptance 9 November 2001;*
*final acceptance 3 April 2002; MS. number: A9148)*

The Argentine ant, *Linepithema humile*, is a widespread invasive species characterized by reduced intraspecific aggression within introduced populations. To illuminate the mechanisms underlying nestmate recognition in Argentine ants, we studied the spatial and temporal fidelity of intraspecific aggression in an introduced population of Argentine ants within which intraspecific aggression does occur. We quantified variation in the presence or absence of intraspecific aggression among nests over time both in the field and under controlled laboratory conditions to gain insight into the role of environmental factors as determinants of nestmate discriminatory ability. In addition, we compared levels of intraspecific aggression between nest pairs to the similarity of their cuticular hydrocarbons to determine the potential role of these compounds as labels for nestmate discrimination. In both field and laboratory comparisons, nest pairs behaved in a consistent manner throughout the course of the experiment: pairs that fought did so for an entire year, and pairs that did not fight remained nonaggressive. Moreover, we found a negative relationship between cuticular hydrocarbon similarity and the degree of aggression between nests, suggesting that these hydrocarbons play a role in nestmate discriminatory ability. In contrast to the prevailing pattern, ants from one site showed a marked change in behaviour during the course of this study. A concomitant change was also seen in the cuticular hydrocarbon profiles of ants from this site.

In social insects, systems for nestmate recognition are often well developed, allowing colony boundaries to be maintained with high fidelity. Because social insect colonies are typically groups of related individuals, nonreproductives gain inclusive fitness by preferentially helping nestmates (Hamilton 1964). Cues used to distinguish nestmates from non-nestmates may be environmentally derived, innate (genetically based), or a combination of both. Using these cues, individuals can discriminate nestmates from non-nestmates, and thus accept or reject individuals they encounter. Environmental odours, such as those acquired from food or nesting material, can be used to assess group membership in many wasps, bees, termites, and some ants (Hölldobler & Michener 1980; Gamboa et al. 1986; Carlin 1989; Hölldobler & Wilson 1990; Breed 1998). Current evidence suggests that genetically based systems are also important, particularly in ants (Breed & Bennett 1987; Hölldobler & Wilson 1990; Banschbach & Herbers 1996; Beye et al. 1998; Stuart & Herbers 2000).

Many insects use cuticular hydrocarbons to recognize mates, conspecifics and colony members (Howard & Blomquist 1982; Vander Meer & Morel 1998). As a result, variation in cuticular hydrocarbons has been used to identify species (Howard et al. 1982; Vander Meer & Lofgren 1990), to differentiate populations (Nowbahari et al. 1990), and to distinguish among different castes within social insect colonies (Howard et al. 1982; Haverty et al. 1996). Intercolonial aggression in ants also correlates with variation in cuticular hydrocarbon profiles (Bonavita-Cougourdan et al. 1987; Nowbahari et al. 1990). Moreover, recent experiments suggest that cuticular hydrocarbons directly affect nestmate recognition in some ant species (Lahav et al. 1999; Thomas et al. 1999; Boulay et al. 2000; Liang & Silverman 2000).

Because ants show a variety of colony structures, they have long been used as model organisms to examine the mechanisms underlying recognition systems (Carlin &

*Correspondence and present address: A. V. Suarez, University of California, Department of Environmental Science, Policy and Management, Division of Insect Biology, 201 Wellman Hall #3112, Berkeley, CA 94720-3112, U.S.A. (email: asuarez@nature.berkeley.edu).*

0003–3472/02/$35.00/0

Hölldobler 1986; Hölldobler & Wilson 1990). In many ants, nestmate discrimination is well developed and workers aggressively defend territories, particularly against conspecifics, resulting in a form of colony structure known as multicoloniality (Hölldobler & Wilson 1977). In contrast, some species show a colony structure known as unicoloniality in which levels of intraspecific aggression are reduced or absent and colony boundaries are weak to nonexistent (Hölldobler & Wilson 1977). Unicoloniality is rare in ants, and is most commonly observed in mound-building wood ants (of the *Formica rufa* group) and many invasive ants, including the Argentine ant, *Linepithema humile* (Hölldobler & Wilson 1990; Passera 1994; Bourke & Franks 1995).

Native to South America, Argentine ants have become established in Mediterranean and subtropical climates throughout the world (Suarez et al. 2001; Tsutsui et al. 2001) where they commonly displace native ants (Bond & Slingsby 1984; Ward 1987; Cammell et al 1996; Human & Gordon 1996; Holway 1998; Suarez et al. 1998). Within native populations, Argentine ants typically appear more multicolonial, with intraspecific aggression frequently occurring over short (<100 m) spatial scales (Suarez et al. 1999; Tsutsui et al. 2000). Within introduced populations, however, intraspecific aggression is almost entirely absent and Argentine ants form expansive supercolonies (Newell & Barber 1913; Markin 1968; Hölldobler & Wilson 1990; Way et al. 1997; Suarez et al. 1999; Tsutsui et al. 2000; Giraud et al. 2002). It has been suggested that unicolonialty has arisen in introduced populations of the Argentine ant as a result of reduced genetic diversity following their introduction (Tsutsui et al. 2000). Introduced populations may not possess the levels of genetic diversity necessary to distinguish nestmates from non-nestmates. This variation in colony structure may also underlie the Argentine's ability to displace native species. Specifically, the numerical advantages key to the Argentine ant's success in displacing native species (Holway 1999; Holway & Case 2001) may result from the reduced mortality, higher foraging rates and greater brood production resulting from an abandonment of costly intraspecific territorial defence (Holway et al. 1998).

Given the lack of intraspecific aggression typical of introduced populations of Argentine ants, and its potential role in their success, it is of great interest to determine the mechanisms responsible for nestmate recognition in this species. Recent work on Argentine ants found a negative relationship between the extent of intraspecific aggression and the degree of genetic similarity between nests in both native and introduced populations (Tsutsui et al. 2000). This finding suggests that nestmate recognition cues are heritable. However, environmental factors also affect nestmate recognition in Argentine ants. For example, acquisition of cuticular hydrocarbons from one prey species, the brown-banded cockroach, *Supella longipalpa*, induces intraspecific aggression between nestmates in a laboratory setting (Liang & Silverman 2000). Such prey-induced aggression can result in colony disassociation after long-term exposure (Silverman & Liang 2001).

In this paper, we examined the temporal fidelity of nestmate recognition among nests of Argentine ants that vary in the degree to which they show intraspecific aggression. We studied changes in behaviour among nests over time in the field and under controlled laboratory conditions in order to gain insight into the role of environmental factors as determinants of nestmate discriminatory ability. In addition, we compared levels of intraspecific aggression between nest pairs to the similarity of their cuticular hydrocarbons to investigate their potential role in nestmate discrimination.

## METHODS

### Selection of Field Sites

To investigate temporal variation in nestmate discriminatory ability in the Argentine ant, we identified nine invaded sites in southern California that varied in the degree to which Argentine ants showed intraspecific aggression. Because intraspecific aggression is rare in the introduced range, extensive surveys throughout southern California were conducted between 1997 and 1999 to identify colonies among which intraspecific aggression occurred (Fig. 1; Suarez et al. 1999; Tsutsui et al. 2000). Colonies from most of these locations have been used previously to investigate the genetic correlates (Tsutsui et al. 2000) and colony-level consequences (Holway et al. 1998) of intraspecific aggression. In August 1999, we collected workers from one nest at each of nine sites and performed pairwise behavioural assays between workers from all the sites ($N$=36 nest pairs). In addition, we collected workers, brood and queens from eight sites to establish laboratory colonies. Each month for 12 months, we returned to each site of collection and repeated behavioural assays among all nest pairs.

### Maintenance of Laboratory Colonies

We established laboratory colonies of Argentine ants from the eight field nests in August 1999, following the protocol of Holway et al. (1998). Each colony occupied a plastic nest container (30 × 14 × 8 cm) lined with Fluon™ (Northern Products Inc., Woonsocket, Rhode Island, U.S.A.) and Tanglefoot™ (The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) to prevent ants from escaping. Each nest container held three test-tubes half-filled with water and plugged with cotton to serve as nest chambers. We started colonies with at least 1000 workers, 100 brood pieces, and a queen to worker ratio similar to that in the field at the time of collection (1–10 queens/laboratory colony). Colonies were fed crickets or scrambled eggs every 3 days and sugar water daily. We performed behavioural assays to measure aggression among all pairwise combinations of the eight nests ($N$=28) each month for 11 months starting 1 month after collection. We also performed behavioural assays each month between the laboratory colonies and the field nest from which they were collected.

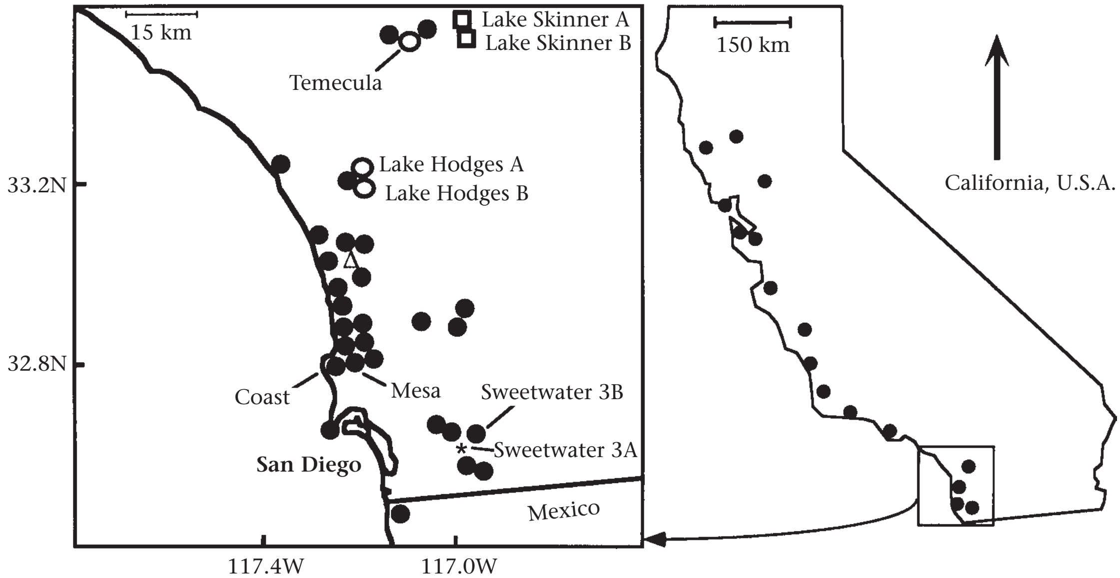

**Figure 1.** Map of California with an inset of southwestern California showing the locations of Argentine ant populations screened for intraspecific aggression. Intraspecific aggression was never detected among sites with the same symbol nor within sites regardless of symbol. The nine field locations used to examine temporal variation in intraspecific aggression in Argentine ants are labelled.

## Behavioural Assays

We quantified intraspecific aggression between nests using a simple behavioural assay (Carlin & Hölldobler 1986; Holway et al. 1998). For each trial, two workers, one from each of two nests, were placed into a 2-dram (3.7 ml) plastic vial coated with Fluon™. We scored behavioural interactions between the two workers for 5 min using the following categories in order of escalating aggression: touch=1 (contacts that included prolonged antennation); avoid=2 (contacts that resulted in one or both ants retreating rapidly in opposite directions); aggression=3 (a brief, <2 s, expression of any of the following behaviours: lunging, biting and pulling legs or antennae, use of chemical defensive compounds); or fight=4 (prolonged aggression between individuals usually resulting in death or dismemberment). At the end of 5 min, we recorded the maximum score attained during the trial; most trials were scored as either a 1 or a 4. The mean value for each set of five trials was used in subsequent analyses.

## Extraction and Isolation of Cuticular Hydrocarbons

Each month, starting in August 1999, we collected 25–100 workers from each field nest and immediately placed them on ice. In the laboratory, we maintained samples at −20 °C until analysis. To extract cuticular hydrocarbons, we washed 5–10 ants from each sample in hexane for 10 min followed by a brief secondary rinse. The solution was loaded onto a silica gel (60–200 mesh, J. T. Baker, Philipsberg, New Jersey, U.S.A.) minicolumn, and hydrocarbons were then eluted with 5 ml of hexane. We analysed hydrocarbon profiles using 30 m=0.32 mm DB-5 capillary columns with an HP589011 or HP5880 GC equipped with a flame-ionization detector and interfaced with a HP GC ChemStation data acquisition system (Rev.A.05.02). Following splitless injection of the sample, we maintained the temperature at 80 °C for 2 min, increased the temperature to 270 °C at 20 °C/min, and then increased the temperature to 310 °C at 3 °C/min. Both detector and injector were maintained at 320 °C.

## Statistical Analyses

Nest pairs fell into two groups at the onset of the experiment: pairs that showed aggression and pairs that did not. While fighting trials were occasionally scored as a 2 (avoiding behaviour) or a 3 (aggressive behaviours present but not sustained), the majority of the trials were scored as either a 1 or 4 (sustained fighting). Subsequently, we considered a nest pair to be aggressive if at least one of the five trials escalated to sustained fighting.

We used a repeated measures multivariate analysis of variance (MANOVA, using JMP, SAS Institute 1996) to compare aggression levels between field and laboratory nest pairs over the 11 months of the study. We conducted four separate MANOVAs: (1) laboratory versus field (aggressive pairs), (2) laboratory versus field (nonaggressive pairs), (3) aggressive versus nonaggressive pairs (field nests), and (4) aggressive versus nonaggressive (laboratory nests). For each MANOVA, dependent variables were aggression scores for each experimental group at each time period.

Numerous studies have reported decreases in intraspecific aggression over time when colonies are reared in the laboratory (Stuart 1987; Breed 1998; Boulay et al. 2000; Chen & Nonacs 2000). To determine whether this occurred in our laboratory colonies, we used regression to assess whether levels of aggression decreased through time for each nest (corrected for multiple comparisons, Rice 1989). To avoid problems of independence that may arise by including each nest pairing in the analysis, we averaged the mean aggression scores across all aggressive pairings each month to use as the dependent variables.

To examine the relationship between cuticular hydrocarbon similarity and intraspecific aggression, we constructed a matrix with each cell containing the pairwise correlation coefficient of hydrocarbon profiles between nests. We used the relative contribution (i.e. height) of the largest 42 peaks as a quantitative measure of each profile. For each nest, we replicated the extraction three to four times, and the mean value for each of the largest 42 peaks was used in subsequent analyses. Each correlation coefficient used in the matrix compared the heights of each of 42 peaks for each nest pair. Second, we constructed a matrix of pairwise aggression scores and used a Mantel test (using Arlequin 2.000; Schneider et al. 2000) with 1 million permutations of the data) to compare the two matrices. Finally, for two field sites (Mesa and Sweetwater 3A) we examined temporal variation in cuticular hydrocarbons by comparing correlation coefficients (as described above) across four time periods from August 1999 to August 2000. These nests were chosen because Sweetwater 3A demonstrated a change in behaviour throughout the course of the study (see Results below). For each comparison, we used individual replicates (rather than means) to determine a mean (± 1 SD) correlation coefficient for each time period. Previous work in this system has documented a relationship between variation in cuticular hydrocarbons and aggression (Liang & Silverman 2000; Silverman & Liang 2001); hydrocarbons with a response time between 20 and 30 min appear most important in determining behavioural variation.

## RESULTS

### Behavioural Patterns

Both in field and laboratory comparisons, the behaviour of nest pairs remained consistent throughout the course of the experiment: aggressive pairs remained aggressive, nonaggressive pairs remained nonaggressive (Fig. 2a). The only exception to this general pattern was the Sweetwater 3A field site. While workers from this site were initially nonaggressive towards ants from a subset of sites (Mesa, Coast and Sweetwater 3B), after January 2000, workers collected from Sweetwater 3A were, in almost all cases, aggressive towards workers from all sites (Fig. 2b, Fig. 3).

MANOVA results confirmed the qualitative patterns in the data. For nests pairs that did not show aggression at the onset of the experiment, there was no significant difference among field or laboratory nest pairs over time (MANOVA: field versus laboratory Wilk's lamda=0.8259, $F_{1,13}=2.7407$, $P=0.1217$; time: Wilk's lamda=0.3567, $F_{10,4}=0.7212$, $P=0.6934$; time × location: Wilk's lamda= 0.4845, $F_{10,4}=0.4255$, $P=0.8755$) (Fig. 2b). This similarity was particularly evident when Sweetwater 3A was removed from the overall analysis (MANOVA: field versus laboratory Wilk's lamda=0.6202, $F_{1,10}=0.0612$, $P=0.9917$) (Fig. 2a). Moreover, laboratory nest pairs that did not show intraspecific aggression at the start of the experiment never consistently developed aggression (Figs 2, 4).

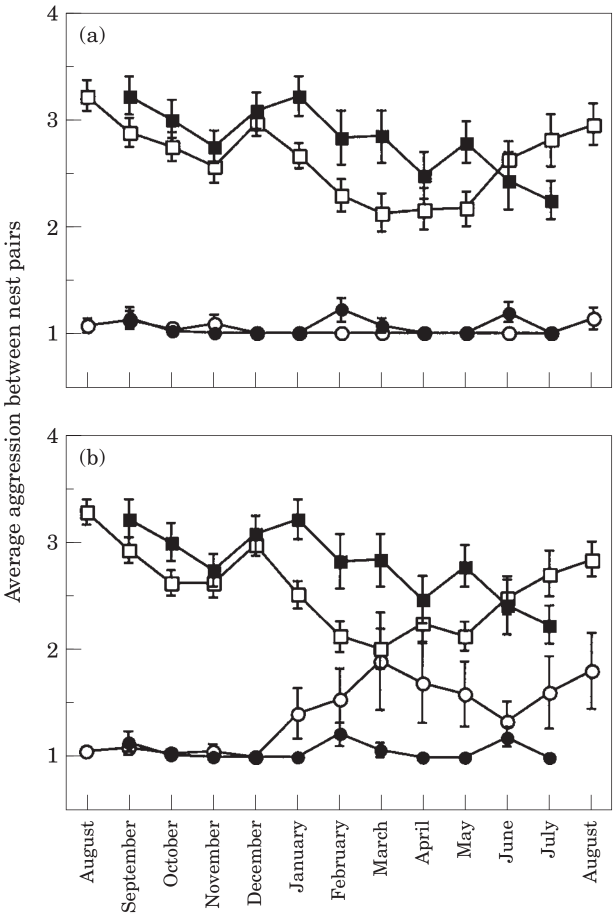

**Figure 2.** Summary of behavioural results for field and laboratory nest pairs. (a) All data for field (open symbols)(not including Sweetwater 3A, see Results) and laboratory (filled symbols) nest pairs categorized into two groups based on the presence (squares) or absence (circles) of aggression between nest pairs at the onset of the study. (b) The same data as in (a) but also including field nest pairs involving Sweetwater 3A demonstrating the change of behaviour of this nest after January. Error bars represent one standard error.

As with nonaggressive pairs, the overall behavioural pattern between aggressive pairs was consistent, despite some temporal variation. Aggressive nest pairs maintained higher assay scores than nonaggressive pairs both for field (MANOVA: Wilk's lamda=0.3672, $F_{1,20}=34.4682$, $P<0.0001$) and laboratory comparisons (MANOVA: Wilk's lamda=0.1997, $F_{1,19}=76.1558$, $P<0.0001$) (Fig. 2). Notably, levels of aggression among laboratory colonies were slightly higher that those in the field (MANOVA: Wilk's lamda=0.8295, $F_{1,26}=5.3431$, $P=0.029$). Among aggressive pairs, levels of aggression varied with time (MANOVA: Wilk's lamda=0.1990, $F_{10,17}=6.8425$, $P=0.0003$), and there was a significant

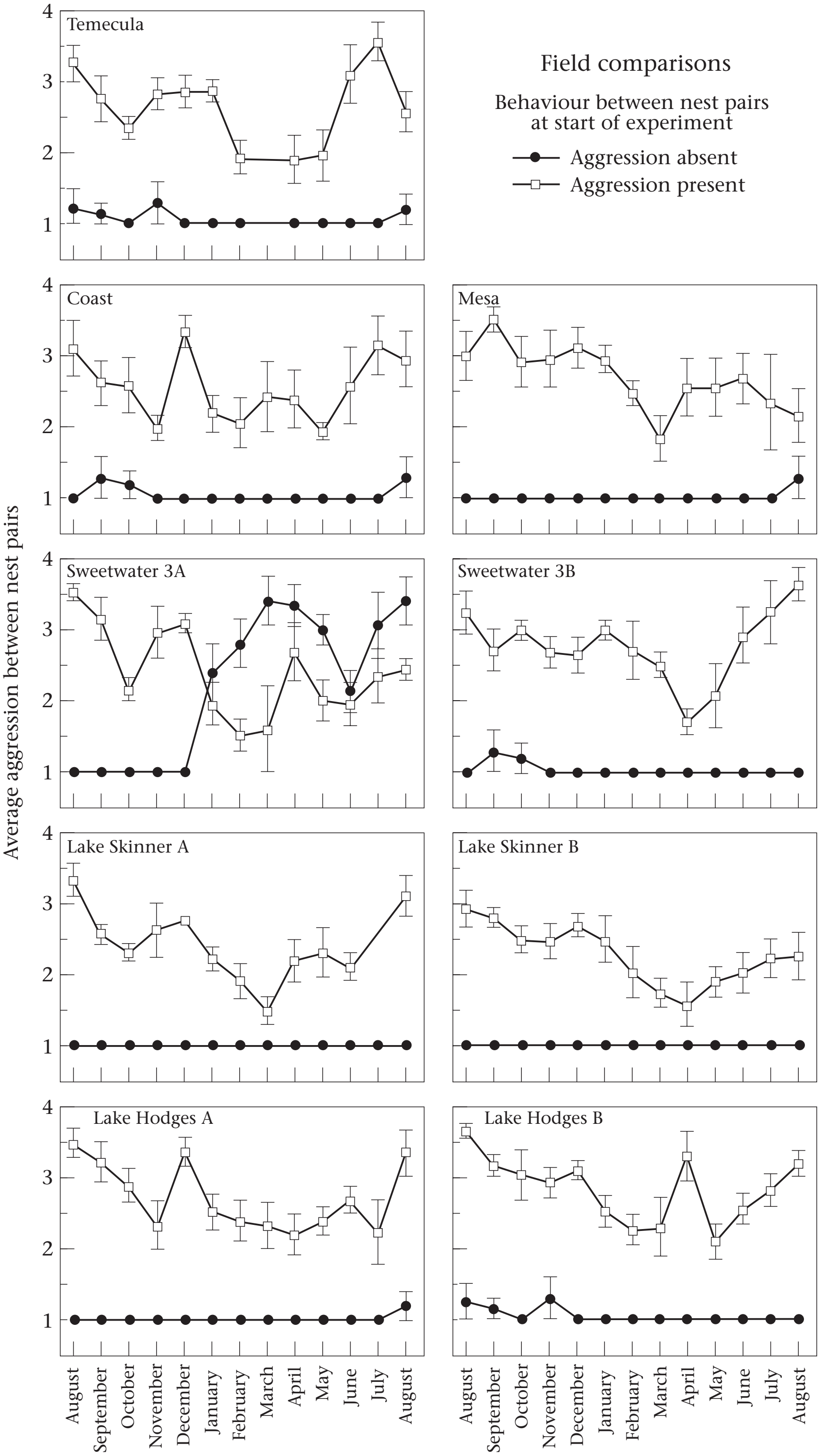

**Figure 3.** Temporal patterns in intraspecific aggression of ants from each field colony when paired with all other sites. Behavioural comparisons between Sweetwater 3A and Mesa, Coast and Sweetwater 3B were excluded after January 2000 (see Results). Missing symbols indicate behavioural assays not conducted for that site during that month. Error bars represent one standard error.

time by location (field or laboratory) interaction (MANOVA: Wilk's lamda=0.3442, $F_{10,17}$=3.2390, $P$=0.0161).

For a majority of nests in the laboratory, aggression, when present, did not decline after 11 months (Fig. 4). However, aggression did decline through time for three

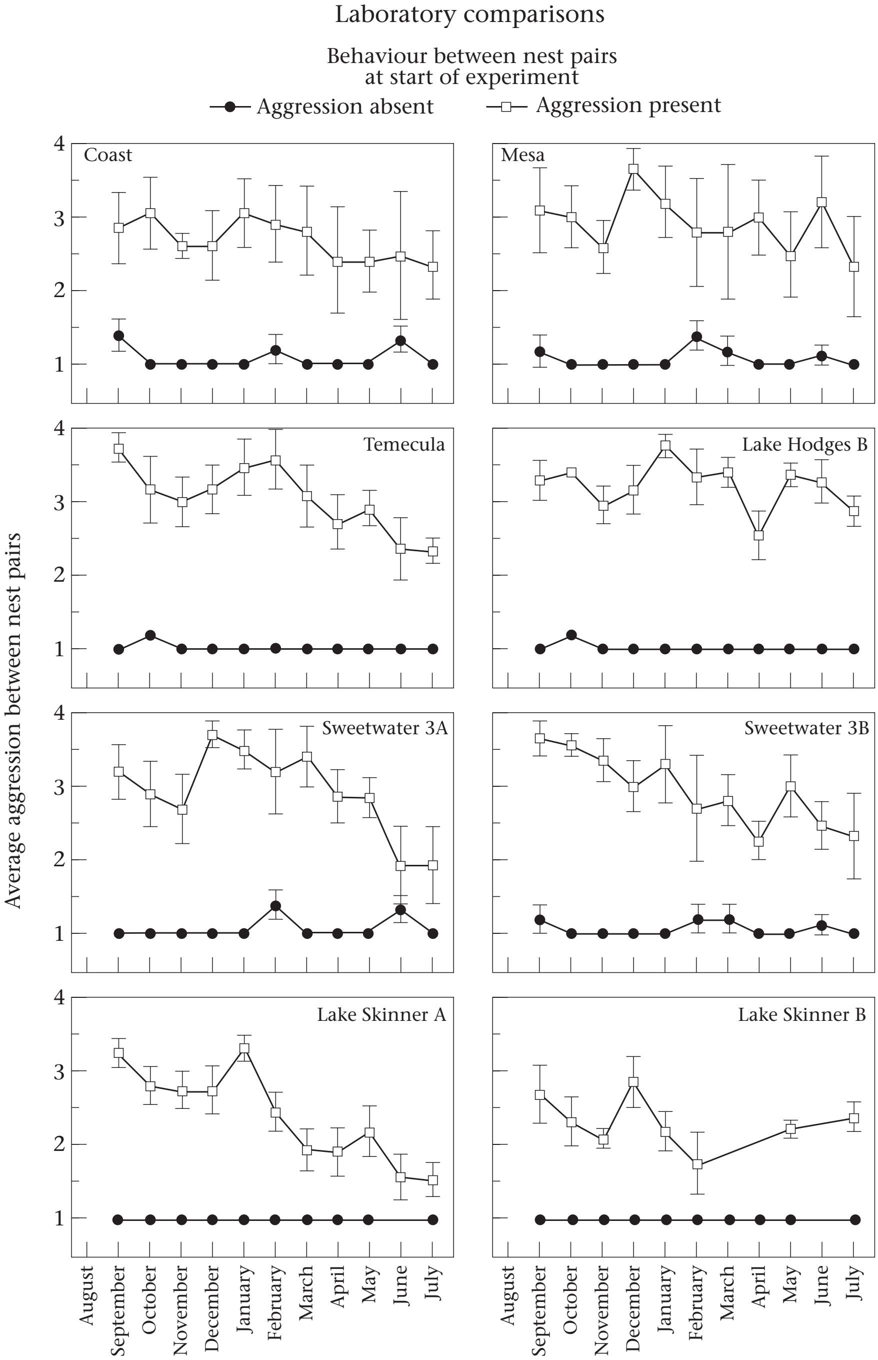

**Figure 4.** Temporal patterns in intraspecific aggression of ants from each laboratory colony when paired with all other sites. Nests from three sites (Temecula, Skinner A and Sweetwater 3B) showed lower levels of aggression at the end of the experiment than at the start. Missing symbols indicate behavioural assays not conducted for that site during that month. Error bars represent one standard error.

nests (Fig. 4): Temecula ($Y=3.704-0.108X$, $R^2=0.611$, $P=0.005$), Skinner A ($Y=3.429-0.165X$, $R^2=0.787$, $P<0.001$), and Sweetwater 3B ($Y=4.004-0.145X$, $R^2=0.634$, $P=0.002$).

### *Field versus laboratory comparisons*

Behavioural assays between laboratory nests and the field sites from which they were collected revealed no aggression until January 2000. At this time, the field and laboratory nests of Sweetwater 3A displayed aggression towards one another (Fig. 5). Aggression was rarely detected in the other comparisons until 10 months into the experiment (Fig. 5). After this time, a few pairings (two of eight nests in June 2000, three of eight nests in July 2000) showed low mean levels of aggression. Across all nests, however, levels of aggression were not higher at the end of the experiment than the beginning (paired *t* test: $t_7=-1.879$, $P=0.1023$).

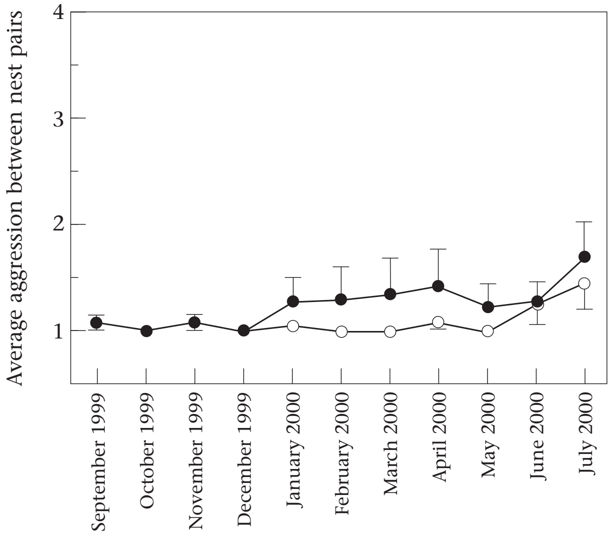

**Figure 5.** Temporal pattern of aggression between laboratory nests and the field nests from which they were collected. Closed circles include nests from all eight locations for which nests were maintained in the laboratory. Open circles exclude data from Sweetwater 3A (see Results). Error bars represent one standard error.

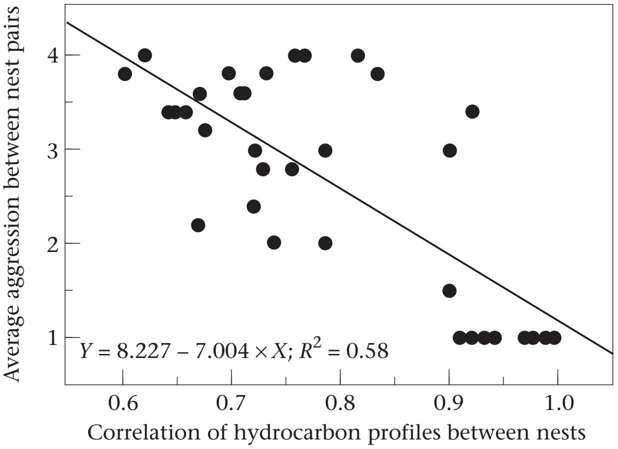

**Figure 6.** Relationship between the average aggression score and the correlation of hydrocarbon profiles between nest pairs. Aggression and hydrocarbon data were collected from field nests in August 1999.

### Hydrocarbon Analysis

Across all nest pairs, levels of aggression were negatively related to the overall similarity of cuticular hydrocarbon profiles (Mantel test: $z$=17.9911, $R^2$=0.5802, $P$<0.0001)(Fig. 6). A specific example of this association is illustrated in Fig. 7. Ants from the Mesa and Sweetwater 3B sites, which never showed aggression towards each other, had similar cuticular hydrocarbon profiles (Pearson's correlation: $r_{42}$=0.994). In contrast, ants from the Temecula site consistently fought with ants from both Mesa and Sweetwater 3B and had profiles that were less correlated (Pearson's correlations: Temecula/Mesa: $r_{42}$=0.711; Temecula/Sweetwater 3B: $r_{42}$=0.778; Fig. 7).

The change in behaviour seen in ants from the Sweetwater 3A field site was associated with a change in the hydrocarbon profiles of those ants. Correlation coefficients between the hydrocarbon profiles from ants collected at Sweetwater 3A in August 1999 and from ants collected at later time points decreased after the change in behaviour. This change was not seen in ants from the Mesa site over the same time period (Fig. 8). Sample profiles from Mesa and Sweetwater 3A also illustrate these differences (Fig. 9).

## DISCUSSION

When initially present, intraspecific aggression between nests of Argentine ants remained high over the course of 1 year, both in the field and under controlled conditions in the laboratory. Likewise, nest pairs that were initially nonaggressive typically did not develop aggression. Taken together, these findings suggest that patterns of nestmate discrimination can remain stable over time, even when environmental factors are tightly controlled. A loss of intraspecific aggression under laboratory conditions is often observed in social insects and is commonly attributed to reduced variation in environmentally derived odours or the necessity of direct contact between nests (Stuart 1987; Breed 1998; Boulay et al. 2000; Chen & Nonacs 2000). However, many studies, including this one, have demonstrated that aggression among nests is maintained or can even increase when controlling for environmental differences in the laboratory (Jutsum et al. 1979; Le Moli et al. 1992; Heinze et al. 1996; Holway et al. 1998; Stuart & Herbers 2000).

Aggressive nest pairs showed significant temporal variation in degree of aggression for both field and laboratory comparisons. This variation may result from a number of factors. First, temporal variation in aggression may be related to extrinsic environmental factors such as temporal changes in diet, temperature or moisture. However, the role of environmental factors in this study appears relatively minor given that levels of aggression were similar between field and laboratory nest pairs for the first 9 months of the study (Fig. 2). Second, temporal variation in aggression may also be related to intrinsic factors such as reproductive phenology (Passera & Aron 1993), changes in local worker density, or variation in within-nest patterns of relatedness. Although such sources of variation undoubtedly influence nestmate recognition, we found no evidence that levels of aggression corresponded with one such measure, the timing of male release (McCluskey 1963; Markin 1970; A. V. Suarez, unpublished data). Variation in aggression may also be related to seasonal cycles of worker production or nest activity (Ichinose 1991). For example, levels of aggression peaked in the autumn when worker densities are highest (Newell & Barber 1913; Markin 1968). Lastly, variation in aggression may be influenced by temporal changes in within-nest relatedness resulting from queen turnover and colony contraction and expansion.

An important exception to the prevailing patterns described above involved the behaviour and hydrocarbon profiles of ants collected at the Sweetwater 3A site.

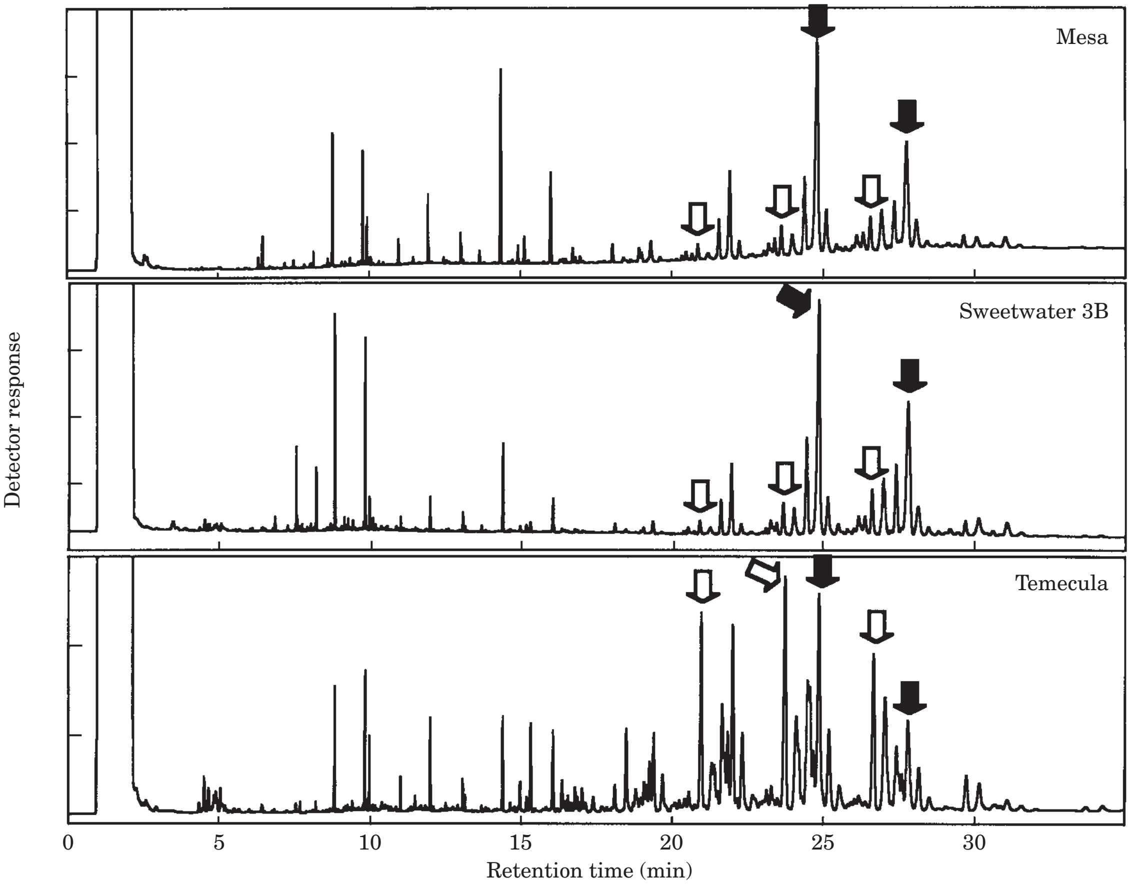

**Figure 7.** Sample cuticular hydrocarbon profiles isolated from Argentine ants collected at the Mesa, Sweetwater 3B and Temecula field sites in August 1999. Ants from Mesa and Sweetwater 3B did not show aggression towards one another, while ants from both locations showed aggression towards ants from Temecula. Closed arrows point to the two largest peaks from the Mesa profile. Open arrows point to example hydrocarbon peaks that differed between Mesa/Sweetwater 3B and Temecula.

At the beginning of the study, workers from this location did not fight against workers from Coast, Mesa and Sweetwater 3B. After 6 months, however, ants from Sweetwater 3A began to fight with ants from these three locations and their laboratory counterparts collected from exactly the same site (Figs 2, 3). Although the mechanism responsible for this result remains unclear, several hypotheses can be advanced. First, the field colony at Sweetwater 3A may have changed its behaviour. For example, a sudden and sustained change in some important environmental factor influencing cuticular hydrocarbon profiles (and subsequently nestmate recognition) may have influenced the behaviour of these ants. A second explanation involves a spatial change in colony boundaries. In this area, two behaviourally antagonistic supercolonies exist in close proximity. It is plausible that during the course of this study the territorial boundaries between these two supercolonies shifted such that the point from which we were sampling at Sweetwater 3A contained workers from one supercolony at the start of the study and workers from the other supercolony after January 2000. If so, we would also expect to see a genetic change in the colony corresponding to the change in behaviour. This interesting and unique event warrants further scrutiny and is the focus of current investigation.

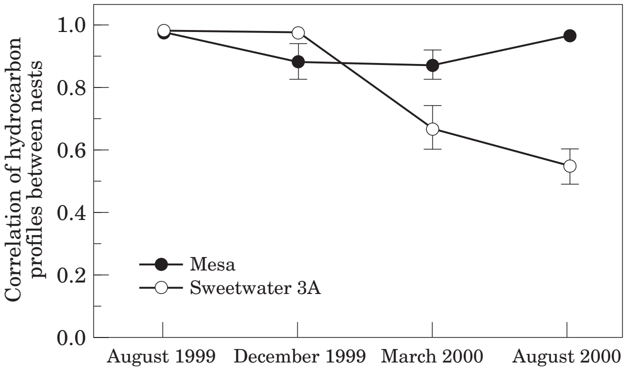

**Figure 8.** Correlation coefficients between hydrocarbon profiles from ants collected in August 1999 and ants collected at three later time periods for the Mesa and Sweetwater 3A sites. Each point represents the mean (+1 SD) correlation coefficient based on three replicates for each time period. The onset of aggression between ants from the Sweetwater 3A field site and their laboratory counterpart occurred in January 2000.

With the exception of Sweetwater 3A, intraspecific aggression rarely developed between laboratory nests and the field nests from which they were collected,

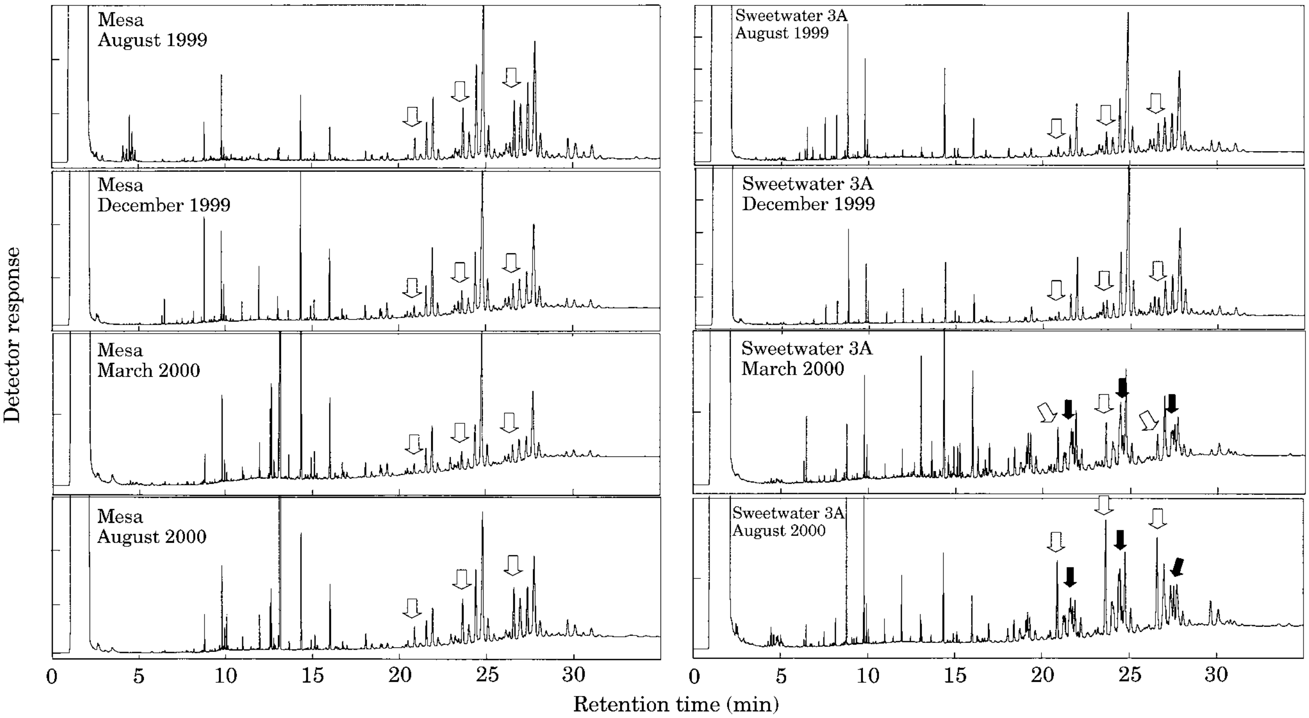

**Figure 9.** Representative hydrocarbon profiles of Argentine ants from Mesa and Sweetwater 3A field sites at four different times during a 1-year period. Open arrows point to the three largest hydrocarbon peaks in ants from Sweetwater 3A during August 2000. These peaks are the same as those identified with open arrows in Fig. 7. Solid arrows point to examples of new hydrocarbon peaks that were only observed in ants from Sweetwater 3A during March and August 2000.

until the last 2 months of the study (Fig. 5). This pattern has several potential explanations. First, environmental determinants of nestmate discrimination may persist under controlled conditions for up to a year. However, given the relatively rapid loss of intraspecific aggression in some laboratory studies (e.g. Stuart 1987; Chen & Nonacs 2000), this explanation seems unlikely. Second, the development of aggression between laboratory nests and their field counterparts at the end of the experiment may have resulted from changes in colony structure associated with the subset of queens that were brought into the laboratory. Because Argentine ant workers typically live less than 1 year (Newell & Barber 1913; Giraud et al. 2002), our laboratory colonies had near complete worker turnover by the end of the experiment. Therefore, long-lasting environmental effects will diminish as field-collected workers are replaced with laboratory-reared workers. Moreover, patterns of within-nest relatedness will change, as field-collected workers are replaced with workers that eclose within the laboratory, reflecting the genetic makeup of the laboratory queens.

As in other ant species, an association exists between cuticular hydrocarbon profiles and aggression in the Argentine ant. Specifically, nest pairs with highly similar hydrocarbon profiles did not show aggression, whereas nest pairs with divergent profiles tended to fight (Fig. 6). The underlying causes of cuticular hydrocarbon variation are poorly understood, although both environmental and genetic factors probably play a role. For example, prey-derived hydrocarbons can cause aggression in Argentine ants (Liang & Silverman 2000). Liang & Silverman (2000) demonstrated that Argentine ant workers can acquire unique hydrocarbons from some prey and that aggression between (and within) nests develops when one nest is fed brown-banded cockroaches. However, nest isolation via prey-derived hydrocarbons may be rare in the field because the diets of individual Argentine ant nests are probably diverse. Moreover, unlike the pronounced response produced by brown-banded cockroaches, aggressive behaviour among nests does not develop when other prey items are used (Liang et al. 2001). Cuticular hydrocarbons may also vary seasonally (Vander Meer et al. 1989; Nielsen et al. 1999), suggesting that either factors related to the environment or reproduction can influence hydrocarbon profiles. Our results from the Mesa site (Fig. 9) suggest that hydrocarbon profiles show little temporal variation throughout the course of a year. However, it would be of great interest to determine in more detail whether temporal variation in aggression is mirrored by temporal variation in hydrocarbon profiles.

The behavioural patterns seen in the laboratory portion of our study suggest that the environmental component of nestmate discrimination may be of minor importance. However, several limitations prevent us from fully exploring the mechanisms of nestmate discriminatory ability in this system. First, increased queen number may compromise the ability of nestmates to recognize foreign individuals (Hölldobler & Wilson

1977) as has been shown in some ants (Keller & Passera 1989; Provost 1989; Starks et al. 1998a; Morel et al. 1990; but see Crosland 1990; Stuart 1991). Argentine ant nests can have hundreds of queens, in both native and introduced populations (A. V. Suarez, N. D. Tsutsui, D. A. Holway, & T. J. Case, personal observations). Ideally, queen number should have been constant in our laboratory study as within-nest relatedness may decrease with increasing queen number, particularly if queens are multiply mated (but see Krieger & Keller 2000). Moreover, aggression between nests may be influenced by intranest relatedness as well as the genetic similarity among nests (Pirk et al. 2001; Tsutsui & Case 2001). Our study also did not examine context-dependent recognition (i.e. proximity to one's colony) (Starks et al. 1998b). Our behavioural assay was performed in a neutral environment in contrast to other studies that place one worker (or a set of workers) in the context of defenders and the other(s) as invaders (e.g. Chen & Nonacs 2000; Stuart & Herbers 2000; Pirk et al. 2001). Directional or asymmetric aggression may be prevalent and important in untangling mechanisms behind nestmate discrimination (Stuart & Herbers 2000; Pirk et al. 2001).

In this study we have attempted to increase our understanding of the underlying causes of intraspecific aggression in a species in which aggression is typically absent from introduced populations. Given that introduced populations of invasive ants often lack intraspecific aggression (Morel et al. 1990; Passera 1994; Holway & Suarez 1999) it will be of great interest to learn more about the nestmate recognition systems of these species (e.g. Obin & VanderMeer 1988). Such information may be useful in the development of control strategies that try to induce intraspecific aggression in invasive ants, either through genetic (Suarez et al. 1999; Tsutsui et al. 2000) or chemical (Silverman & Liang 2001) means.

## Acknowledgments

We would like to thank J. Baez and K. Royer for help with the aggression assays. The manuscript was improved greatly by comments from Jules Silverman, Robin Stuart and two anonymous referees. This work was supported by the Miller Institute for Basic Research in Science and grants from the United States Department of Agriculture NRICGP to A.V.S. (00–35302–9417) and D.A.H. (99–35302–8675), and the National Science Foundation to T.J.C. (DEB 9610306).

## References

**Banschbach, V. S. & Herbers, J. M.** 1996. Complex colony structure in social insects: I. Ecological determinants and genetic consequences. *Evolution*, **50,** 285–297.

**Beye, M., Neumann, P., Chapuisat, M., Pamilo, P. & Moritz, R. F. A.** 1998. Nest mate recognition and the genetic relatedness of nests in the ant *Formica pratensis. Behavioral Ecology and Sociobiology*, **43,** 67–72.

**Bonavita-Cougourdan, A., Clement, J. L. & Lange, C.** 1987. Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus*. Scop. *Journal of Entomological Science*, **22,** 1–10.

**Bond, W. & Slingsby, P.** 1984. Collapse of an ant–plant mutualism: the Argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. *Ecology*, **65,** 1031–1037.

**Boulay, R., Hefetz, A., Soroker, V. & Lenoir, A.** 2000. *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Animal Behaviour*, **59,** 1127–1133.

**Bourke, A. F. G. & Franks, N. R.** 1995. *Social Evolution in Ants*. Princeton, New Jersey: Princeton University Press.

**Breed, M. D.** 1998. Recognition phermones of the honey bee. *BioScience*, **48,** 463–470.

**Breed, M. D. & Bennett, B.** 1987. Kin recognition in highly eusocial insects. In: *Kin Recognition in Animals* (Ed. by D. J. C. Fletcher & C. D. Michener), pp. 243–285. New York: J Wiley.

**Cammell, M. E., Way, M. J. & Paiva, M. R.** 1996. Diversity and structure of ant communities associated with oak, pine, eucalyptus and arable habits in Portugal. *Insectes Sociaux*, **43,** 37–46.

**Carlin, N. F.** 1989. Discrimination between and within colonies of social insects: two null hypotheses. *Netherlands Journal of Zoology*, **39,** 86–100.

**Carlin, N. F. & Hölldobler, B.** 1986. The kin recognition system of carpenter ants (*Camponotus* spp.); I. Hierarchical cues in small colonies. *Behavioral Ecology and Sociobiology*, **19,** 123–134.

**Chen, J. S. C. & Nonacs, P.** 2000. Nestmate recognition and intraspecific aggression based on environmental cues in Argentine ants (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **93,** 1333–1337.

**Crosland, M. W. J.** 1990. The influence of queen, colony size, and worker ovarian development on nest mate recognition in the ant *Rhytidoponera confusa. Animal Behaviour*, **39,** 413–425.

**Gamboa, G. J., Reeve, H. K. & Pfennig, D. W.** 1986. The evolution and ontogeny of nestmate recognition in social wasps. *Annual Review of Entomology*, **31,** 431–454.

**Giraud, T., Pederson, J. S. & Keller, L.** 2002. Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences, U.S.A.*, **99,** 6075–6079.

**Hamilton, W. D.** 1964. The genetical evolution of social behavior. I and II. *Journal of Theoretical Biology*, **7,** 1–52.

**Haverty, M. I., Grace, J. K., Nelson, L. J. & Yamamoto, R. T.** 1996. Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Coptotermes formosanus* Shiraki (Isoptera: Phinotermitidae). *Journal of Chemical Ecology*, **22,** 1813–1834.

**Heinze, J., Foitzik, S., Hippert, A. & Holldobler, B.** 1996. Apparent dear enemy phenomenon and environmental-based recognition cues in the ant *Leptothorax nylanderi. Ethology*, **102,** 510–522.

**Hölldobler, B. & Michener, C. D.** 1980. Mechanisms of identification and discrimination in social hymenoptera. In: *Evolution of Social Behavior: Hypotheses and Empirical Tests* (Ed. by H. Markl), pp. 35–58. Weinheim: Verlag Chemie.

**Hölldobler, B. & Wilson, E. O.** 1977. The number of queens: an important trait in ant evolution. *Naturwissenschaften*, **64,** 8–15.

**Hölldobler, B. & Wilson, E. O.** 1990. *The Ants*. Cambridge, Massachusetts: Harvard University Press.

**Holway, D. A.** 1998. Effects of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oecologia*, **116,** 252–258.

**Holway, D. A.** 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology*, **80,** 238–251.

**Holway, D. A. & Case, T. J.** 2001. Effects of colony-level variation on competitive ability in the invasive Argentine ant. *Animal Behaviour*, **61,** 1181–1192.

**Holway, D. A. & Suarez, A. V.** 1999. Animal behavior: an essential component of invasion biology. *Trends in Ecology and Evolution*, **14,** 328–330.

**Holway, D. A., Suarez, A. V. & Case, T. J.** 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, **281,** 949–952.

**Howard, R. W. & Blomquist, G. J.** 1982. Chemical ecology and biochemistry of insect hydrocarbons. *Annual Review of Entomology*, **27,** 149–172.

**Howard, R. W., McDaniel, C. A., Nelson, D. R., Blomquist, G. J., Gelbaum, L. T. & Zalkow, L. H.** 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species- and caste-recognition cues. *Journal of Chemical Ecology*, **8,** 1227–1239.

**Human, K. G. & Gordon, D. M.** 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia*, **105,** 405–412.

**Ichinose, K.** 1991. Seasonal variation in nestmate recognition in *Paratrechina flavipes* (Smith) worker ants (Hymenoptera: Formicidae). *Animal Behaviour*, **41,** 1–6.

**Jutsum, A. R., Saunders, T. S. & Cherrett, J. M.** 1979. Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. *Animal Behaviour*, **27,** 839–844.

**Keller, L. & Passera, L.** 1989. Influence of the number of queens on nestmate recognition and attractiveness of queens to workers in the Argentine ant, *Iridomyrmex humilis* (Mayr). *Animal Behaviour*, **37,** 733–740.

**Krieger, M. J. B. & Keller, L.** 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. *Molecular Ecology*, **9,** 119–126.

**Lahav, S., Soroker, V., Hefetz, A. & Vander Meer, R. K.** 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften*, **86,** 246–249.

**Le Moli, F., Mori, A. & Grasso, D. A.** 1992. Nestmate and conspecific non-nestmate recognition in *Formica cunnicularia* Latr.: the effect of diet difference. In: *Biology and Evolution of Social Insects* (Ed. by J. Billen), pp. 161–165. Leuven, Belgium: Leuven University Press.

**Liang, D. & Silverman, J.** 2000. 'You are what you eat': diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant *Linepithema humile. Naturwissenschaften*, **87,** 412–416.

**Liang, D., Bloomquist, G. J. & Silverman, J.** 2001. Hydrocarbon-release nestmate aggression in the Argentine ant, *Linepithema humile*, following encounters with insect prey. *Comparative Biochemistry and Physiology, Part B*, **129,** 871–882.

**McCluskey, E. S.** 1963. Rhythms and clocks in harvester and Argentine ants. *Physiological Zoology*, **36,** 273–292.

**Markin, G. P.** 1968. Nest relationship of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae). *Journal of the Kansas Entomological Society*, **41,** 511–516.

**Markin, G. P.** 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae) in southern California. *Annals of the Entomological Society of America*, **63,** 1238–1242.

**Morel, L., Vandermeer, R. K. & Lofgren, C. S.** 1990. Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **83,** 642–647.

**Neilsen, J., Boomsma, J. J., Oldham, N. J., Petersen, H. C. & Morgan, E. D.** 1999. Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum. Insectes Sociaux*, **46,** 58–65.

**Newell, W. & Barber, T. C.** 1913. *The Argentine Ant*. Washington, D.C.: USDA Bureau of Entomology Bulletin Number 122.

**Nowbahari, E., Lenoir, A., Clement, J. L., Lange, C., Bagneres, A. G. & Joulie, C.** 1990. Individual, geographical and experimental variation of cuticular hydrocarbon of the ant *Cataglyphis cursor* (Hymenoptera:Formicidae): their use in nest and subspecies recognition. *Biochemical Systematics Ecology*, **18,** 63–73.

**Obin, M. S. & Vander Meer, R. K.** 1988. Sources of nestmate recognition cues in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Animal Behaviour*, **36,** 1361–1370.

**Passera, L.** 1994. Characteristics of tramp species. In: *Exotic Ants: Biology, Impact, and Control of Introduced Species* (Ed. by D. F. Williams), pp. 23–43. Boulder, Colorado: Westview Press.

**Passera, L. & Aron, S.** 1993. Social control over the survival and selection of winged virgin queens in an ant without nuptial flights: *Iridomyrmex humilis*. *Ethology*, **93,** 225–235.

**Pirk, C. W. W., Neumann, P., Moritz, R. F. A. & Pamilo, P.** 2001. Intranest relatedness and nestmate recognition in the meadow ant *Formica pratensis* (R.). *Behavioral Ecology and Sociobiology*, **49,** 366–374.

**Provost, E.** 1989. Social environmental factors influencing mutual recognition of individuals in the ant *Leptothorax lichtensteini* Bondr. (Hymenoptera: Formicidae). *Behavioural Processes*, **18,** 35–59.

**Rice, W. R.** 1989. Analyzing tables of statistical tests. *Evolution*, **43,** 223–225.

**SAS Institute.** 1996. *JMP*. version 3.2. Cary, North Carolina: SAS Institute.

**Schneider, S., Roessli, D. & Excoffier, L.** 2000. *Arlequin. Version 2.000: a Software for Population Genetic Anaylsis.* Geneva: Genetics and Biometry Laboratory, University of Geneva.

**Silverman, J. & Liang, D.** 2001. Colony disassociation following diet partitioning in a unicolonial ant. *Naturwissenschaften*, **88,** 73–77.

**Starks, P. T., Watson, R. E., Dipaola, M. J. & Dipaola, C. P.** 1998a. The effect of queen number on nestmate discrimination in the facultatively polygynous ant *Pseudomyrmex pallidus* (Hymenoptera: Formicidae). *Ethology*, **104,** 573–584.

**Starks, P. T., Fischer, D. J., Watson, R. E., Melikian, G. L. & Nath, S. D.** 1998b. Context-dependent nestmate discrimination in the paper wasp, *Polistes dominulus*: a critical test of the optimal acceptance threshold model. *Animal Behaviour*, **56,** 449–458.

**Stuart, R. J.** 1987. Transient nest mate recognition cues contribute to a multicolonial population structure in the ant *Leptothorax curvispinosus*. *Behavioral Ecology and Sociobiology*, **21,** 229–235.

**Stuart, R. J.** 1991. Nestmate recognition in leptothoracine ants: testing for effects of queen number, colony size, and species of intruder. *Animal Behaviour*, **42,** 277–284.

**Stuart, R. J. & Herbers, J. M.** 2000. Nestmate recognition in ants with complex colonies: within and between population variation. *Behavioral Ecology*, **11,** 676–685.

**Suarez, A. V., Bolger, D. T. & Case, T. J.** 1998. The effect of fragmentation and invasion on the native ant community in coastal southern California. *Ecology*, **79,** 2041–2056.

**Suarez, A. V., Tsutsui, N. D., Holway, D. A. & Case, T. J.** 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions*, **1,** 43–53.

**Suarez, A. V., Holway, D. A. & Case, T. J.** 2001. Predicting patterns of spread in biological invasions dominated by jump dispersal: lessons from Argentine ants. *Proceedings of the National Academy of Sciences U.S.A.*, **98,** 1095–1100.

**Thomas, M. L., Parry, L. J., Allen, R. A. & Elgar, M. A.** 1999. Geographic affinity, cuticular hydrocarbons and colony recognition in the Australian meat ant *Iridomyrmex purpureus. Naturwissenschaften*, **86,** 87–92.

**Tsutsui, N. D. & Case, T. J.** 2001. Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution*, **55,** 976–985.

**Tsutsui, N. D., Suarez, A. V., Holway, D. A. & Case, T. J.** 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, U.S.A.*, **97,** 5948–5953.

**Tsutsui, N. D., Suarez, A. V., Holway, D. A. & Case, T. J.** 2001. Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. *Molecular Ecology*, **10,** 2151–2161.

**Vander Meer, R. K. & Lofgren, C. S.** 1990. Chemotaxonomy applied to fire ant systematics in the United States and South America. In: *Applied Myrmecology: a World Perspective* (Ed. by R. K. Vander Meer, K. Jaffe & A. Cedeno), pp. 75–84. Boulder, Colorado: Westview Press.

**Vander Meer, R. K. & Morel, L.** 1998. Nestmate recognition in ants. In: *Pheromone Communication in Social Insects* (Ed. by R. K. Vander Meer, M. D. Breed, K. E. Espelie & M. L. Winston), pp. 79–103. Boulder, Colorado: Westview Press.

**Vander Meer, R. K., Saliwanchik, D. & Lavine, B.** 1989. Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*: implications for nestmate recognition. *Journal of Chemical Ecology*, **15,** 2115–2125.

**Ward, P. S.** 1987. Distribution of the introduced Argentine ant (*Iridomyrmex humilis*) in natural habitats of the lower Sacramento Valley and its effects on the indigenous ant fauna. *Hilgardia*, **55,** 1–16.

**Way, M. J., Cammell, M. E., Paiva, M. R. & Collingwood, C. A.** 1997. Distribution and dynamics of the Argentine ant *Linepithema* (*Iridomyrmex*) *humile* (Mayr) in relation to vegetation, soil conditions, topography and native competitor ants in Portugal. *Insectes Sociaux*, **44,** 415–433.