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BIOCHEMICAL AND BEHAVIORAL EVIDENCE FOR HYBRIDIZATION BETWEEN FIRE ANTS, *Solenopsis invicta* AND *Solenopsis richteri* (HYMENOPTERA: FORMICIDAE)

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Abstract—Behavioral and biochemical evidence is presented for hybridization between the fire ants, *Solenopsis richteri* and *S. invicta.* The response of the two species to extracts of their trail pheromones presented as a point source is clearly species-specific; however, hybrid workers responded to parental Dufour's gland extracts and parental workers responded to Dufour's gland extracts of the hybrid. The behavioral evidence for hybridization was confirmed by gas chromatograph comparison of the Dufour's gland extracts of the three fire ant forms, which showed a pattern for the hybrid that was intermediate to the two parental species.

Key Words—Fire ants, *Solenopsis invicta, Solenopsis richteri,* Hymenoptera, Formicidae, trail pheromone, hybridization.

INTRODUCTION

The discovery of hybridization between two imported fire ant species, *Solenopsis invicta* and *S. richteri,* in the United States has raised questions regarding the validity of their species status and the mechanism by which the extensive hybrid population is being maintained (Vander Meer et al., 1985; Ross et al., 1987; Vander Meer and Lofgren, 1988). Based on the earliest known records of imported fire ants in the United States (see Lofgren, 1986), it was deduced that a black form entered the Mobile, Alabama, area around 1918. A red form was also discovered in the Mobile area and was thought to have been introduced

in the 1930s. Both forms were identified as *S. saevissima* var. *richteri* until Buren (1972) elevated the black form to species status (*S. richteri*) and described the red form as a new species (*S. invicta*). These assignments were based on their lack of hybridization and the consistency of phenetic characters in the United States populations. However, hybridization between the two species has been recently discovered on the basis of biochemical characters (Vander Meer et al., 1985). Morphologically the hybrid is difficult to distinguish from *S. richteri* (Vander Meer et al., 1985; Ross et al., 1987). Biochemical analysis of museum specimens collected in the United States (Vander Meer and Lofgren, in preparation) indicates that hybridization has occurred wherever the range of the two forms has overlapped. Although this indicates that the criteria for differentiating them as species may no longer be valid, we will continue to use the species designations of Buren (1972).

The rapid spread of imported fire ants after their introduction was due primarily to their transportation by man in nursery stock and sod. The speed and mode of this spread resulted in an early disjunct distribution in Alabama, Louisiana, and Mississippi (see Bruce et al., 1949; Culpepper, 1953), which could not be explained by dispersion after natural mating flights. Currently the gaps have been filled, except at the western and northern frontiers of their range. At the present time, imported fire ants occupy 100,000,000 hectares in 11 states of the south and southeast and in Puerto Rico. Unfortunately, previous surveys of fire ant infestations did not distinguish between the red and black forms, so we know very little of the population dynamics of the two forms that have taken place since the 1940s.

Species-specific pheromones in social insects often play an important role in maintaining species isolation, both sexually and in foraging situations (Hölldobler and Carlin, 1987). In addition, the competitiveness of a colony or ant population is mediated to a large extent by its ability to discriminate the chemical cues associated with territory, recruitment, and competitor/enemy recognition (reviewed in Hölldobler and Carlin, 1987). In this paper we compare the behavioral species specificity of the recruitment pheromones produced by *S. invicta, S. richteri* and their hybrid, as well as the biochemical composition of these pheromones.

METHODS AND MATERIALS

Source of Ant Colonies. All individuals used as sources of Dufour's glands and for bioassays were taken from mature (producing sexuals) queenright, monogynous colonies. *Solenopsis invicta* colonies were collected from the field or reared from newly mated queens collected in Alachua County, Florida (see Banks et al., 1981, for methodology). Queenright *Solenopsis richteri* colonies

were collected along the Natchez Trace in Lee County, Mississippi. Hybrid colonies were collected from Lowndes County, Mississippi. The identification of the *S. richteri* and hybrid colonies was verified by gas chromatographic analyses of venom alkaloids and cuticular hydrocarbons (Vander Meer et al., 1985).

Source of Glandular Trail Pheromone Extracts. Dufour's glands from randomly chosen worker ants from each of the *Solenopsis* forms were extirpated in water, then transferred to, and macerated in a vial containing hexane (Burdick and Jackson, HPLC grade, Muskegan, Michigan). Pooled Dufour's gland extracts were used for both gas chromatographic (GC) analysis and bioassays. For bioassays, the concentration of the Dufour's gland extract was adjusted to give the desired number of worker equivalents (WE) per microliter by dilution with hexane or evaporation under a stream of nitrogen.

Gas Chromatograph Analysis. Hexane extracts or soaks were analyzed by GC on a Varian 3700 gas chromatograph (Sunnyvale, California), equipped with a flame ionization detector. Analysis of hexane soaks (1–24 hr) for venom alkaloids and cuticular hydrocarbons was performed with a 30-m DB-1 fused silica column (J&W Scientific, Inc., Folsom, California) and oven program of 150°C to 285°C at 5°/min (Vander Meer et al., 1985; Ross et al., 1987). Separation of the volatile components of the Dufour's gland extracts was achieved with a 15-m DB-1 fused silica column (J&W Scientific), operated isothermal at 88°C. Peak areas were integrated and the separation visualized on a Varian Vista 401 data processor.

Recruitment Pheromone Point Source Bioassay. One to three rearing cells from queenright laboratory-reared colonies were placed on top of each other in the center of a clean colony tray (7 × 44 × 56 cm). Ten positions were marked in a circle, 15 cm from the edge of the cells and equidistant from each other around the cells. Workers in the foraging arena of the donor colony tray were also transferred to the clean bioassay tray. The ants were given a minimum of 1 hr to habituate to their new surroundings. Squares of blotter paper (2 × 2 cm) on which a 1.5-cm-diameter circle had been drawn in the center were placed on larger squares of aluminum foil. The circular area was treated with a Dufour's gland extract (5 μl of hexane) containing one worker equivalent of one of the following: Dufour's gland extracts from *S. invicta, S. richteri,* or the hybrid (as defined by GC analysis; see Vander Meer et al., 1985; Ross et al., 1987), or a hexane control. The samples were randomly placed on the numbered locations around the colony cells. The number of ants within the 1.5-cm circle on the blotter paper squares were counted at 5-min intervals for 30 min. The sum of the six counts was regarded as the result for each position. Each bioassay was replicated using 10 different *S. invicta, S. richteri,* and hybrid colonies. Colony-to-colony variation was leveled out by setting the response of test colony workers to their own Dufour's gland extract at 100 and their response to the hexane control at 0 (see also Vander Meer et al., 1988).

RESULTS

Point-Source Bioassay. The response of foraging worker ants in the point-source bioassay to Dufour's gland extracts from *S. invicta, S. richteri,* and the hybrid are shown in Table 1. *S. invicta* responded poorly to the Dufour's gland extract of *S. richteri* and, similarly, *S. richteri* responded minimally to the Dufour's gland extract of *S. invicta,* demonstrating distinct behavioral differences for the two fire ant species in this bioassay. The response of workers from both parental species to hybrid Dufour's gland extracts was indistinguishable from the response of hybrid workers to their own Dufour's gland extract. Likewise, hybrid workers did not discriminate between the Dufour's gland extracts of the parental species or extracts of their own Dufour's glands.

Chemical Analysis of Dufour's Gland Extracts. The results of gas chromatographic analysis of Dufour's gland extracts from *S. invicta, S. richteri,* and hybrid are shown in Figure 1. The chromatographic trace of *S. invicta* has the characteristic profile previously reported (Vander Meer et al., 1981). (*Z,E*)-alpha-farnesene (A) is the major peak followed by an as yet unidentified monounsaturated tricyclic homosesquiterpene (B) (Vander Meer et al., 1988) and two known acyclic homosesquiterpenes (C and D) (Alvarez et al., 1987). The *S. richteri* profile is characterized by a single major component that has been shown to be identical to component B in *S. invicta.* However, none of the minor components in the *S. richteri* GC trace correspond to the identified biologically active components observed for *S. invicta.* The Dufour's gland GC trace of the hybrid shows a combination of all four known biologically active recruitment pheromones (A, B, C, and D) in proportions indicative of a blend of the two parental species. Components A, C, and D, absent from the Dufour's glands of *S. richteri,* are present in the hybrid, and component B, which is found in only trace amounts from Dufour's glands of *S. invicta,* is conspicuously present in the GC trace of the Dufour's gland of the hybrid.

TABLE 1. PERCENT RESPONSE OF FIRE ANT WORKERS IN POINT-SOURCE BIOASSAY OF DUFOUR'S GLAND EXTRACTS[a]

Test species	Dufour's gland extract[b]		
	S. invicta	*S. richteri*	Hybrid
S. invicta	100.0	32.1 ± 7.7	123.3 ± 16.2
S. richteri	16.1 ± 1.8	100.0	103.2 ± 7.3
Hybrid	109.1 ± 18.9	147.3 ± 17.2	100.0

[a] Results are expressed as the mean and standard error of 10 replicates.
[b] Test samples were applied at a concentration of 1 WE/5 μl solution.

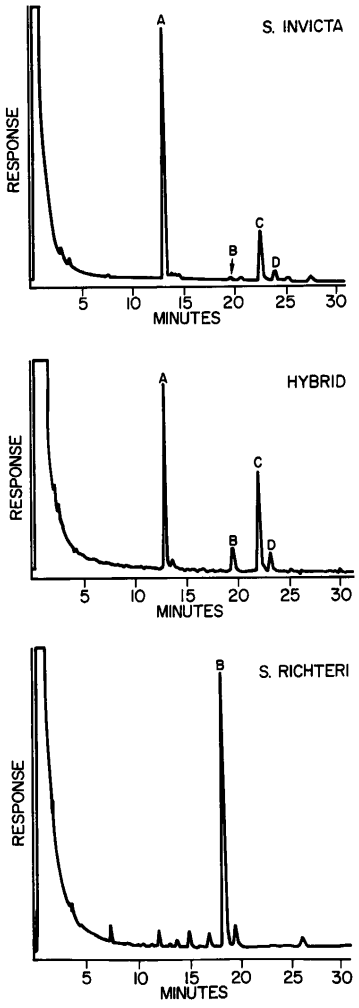

FIG. 1. Gas chromatograms of the volatile region from Dufour's gland extracts that have recruitment pheromone activity. See Methods and Materials section for details of the GC conditions.

DISCUSSION

Species-specific semiochemicals play an important role in many facets of social insect behavior, for example, reproductive isolation in bumblebees (van Honk et al., 1978) and recruitment of worker ants to food resources (Morgan, 1984; Attygale and Morgan, 1985). In some ant species, colony-specific cues (*Lasius*

neoniger) (Traniello, 1980) and even individual-specific orientation trails (*Pachycondyla tesserinoda*) (Jessen and Maschwitz, 1986) have been discovered. Although nonsemiochemical factors also affect species separation, the partitioning of resources in complex ant communities is largely dependent on chemical communication employed in territorial and recruitment behavior (Hölldobler and Carlin, 1987).

Fire ants have a complex food foraging and recruitment mechanism. Following food discovery, the foragers use light as a visual orientation cue to guide them to the entrance to their foraging tunnel. On the return trip, the forager leaves a trail by touching its stinger to the substrate and simultaneously releasing pheromone that originates in the Dufour's gland (Wilson 1959, 1962; Vander Meer, 1986). At the nest, additional pheromone is released, which induces other workers to follow the trail (Vander Meer, 1986; Vander Meer et al., unpublished). Depending on the size and quality of the food source, the returning workers reinforce the trail, thus providing a feedback mechanism regulating the number of workers recruited (Wilson, 1962).

The fire ant recruitment process has been reduced to three measurable and sequential behaviors: (A) attraction, (B) orientation induction, and (C) orientation (Vander Meer, 1986). Orientation has been demonstrated to be nonspecific for the two species (Barlin et al., 1976; Jouvenaz et al., 1978). Orientation induction shows an asymmetric specificity (Vander Meer, 1986; Vander Meer and Lofgren, unpublished). However, worker response to a point source of Dufour's gland extract is species-specific (Vander Meer, 1986). The latter bioassay measured attraction and aggregation to Dufour's gland extracts (Vander Meer et al., 1988). Quantitative differences in active components present in the Dufour's glands of the two species (Figure 1), coupled with differential worker sensitivity to those components, could account for the observed nonspecificity of the orientation and attraction bioassays (Barlin et al., 1976; Jouvenaz et al., 1978), as well as the species specificity of the point-source bioassay (Vander Meer, 1986).

Our results using the species-specific point-source bioassay mentioned above (Table 1) clearly provides the first behavioral evidence for hybridization between *S. invicta* and *S. richteri*. Although the chemistry associated with the three recruitment behavioral subcategories is not fully known, it is clear from a visual comparison of the gas chromatograph traces (Figure 1) of the hybrid and its parents that the hybrid combines the chemical features of its parents. This is analogous to the gas chromatograph profiles of the hybrid and parent venom alkaloids and hydrocarbons that were first used to describe the hybrid (Vander Meer et al., 1985; Ross et al., 1987).

Recent surveys in Mississippi, Alabama, and Georgia indicate that the range of the hybrid population is extensive throughout the northern portions of

these states (Vander Meer and Lofgren, 1988; Diffie et al., 1988). The only place that the hybrid is sandwiched between its two parental types is in northeastern Mississippi. At the present time competition for territory is taking place between (1) hybrid and *S. invicta;* (2) hybrid and *S. richteri;* and (3) hybrid and hybrid, but not between the two parental types.

Chemical analyses of fire ant samples from the Meridian, Mississippi, area over a 24-year period of time indicates that the hybrid and *S. invicta* have reached a point of equilibrium, with neither form capable of displacing the other (Vander Meer and Lofgren, unpublished). In contrast, chemical analyses at the hybrid–*S. richteri* interface indicate that the hybrid is displacing *S. richteri.* It is probably only a matter of time before *S. richteri* is eliminated from the United States.

Analysis of alcohol-preserved fire ant specimens for their venom alkaloid patterns demonstrated that hybridization between *S. invicta* and *S. richteri* was taking place wherever and whenever the two species have met (Vander Meer and Lofgren, in preparation). Consequently, all three forms have been vying for territory over the last 50 years. It could be argued that the lack of recruitment specificity of the hybrid would give it a homospecific versus a heterospecific advantage in resource competition with either of the parent species. However, the current distribution suggests that other factors, such as environmental and ecological adaptability, have probably played an important role in the observed population dynamics of *S. invicta, S. richteri,* and their hybrid. In South America, *S. invicta* occupies the northern tropical and subtropical areas, whereas *S. richteri* occupies the southern, more temporate climatic zone (Buren, 1972). The same climatic distribution is currently seen for the two parental types in the United States. The hybrid occupies a climatic niche in between their parents. To arrive at the present hybrid distribution requires: (1) a fitness advantage of *S. invicta* over hybrid in the southern parts of their United States distribution; (2) a fitness advantage of the hybrid over *S. invicta* in the northern parts of their distribution; (3) fitness factors being equal, no competitive advantage for *S. invicta* or the hybrid; and (4) environmental factors being equal, a possible competitive advantage of hybrid over *S. richteri.*

Our behavioral studies demonstrate for the first time hybridization between *S. invicta* and *S. richteri* through a behavioral bioassay and again through a species-specific biochemical character (Vander Meer et al., 1985). This work brings us closer to an understanding of possible mechanisms by which the two fire ant species and their hybrid reached their present distribution in the United States.

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