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Chemical Taxonomy as a Tool for Separating *Solenopsis* spp.

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Chemotaxonomy had its beginnings in 1886 when Helen Abbott published a paper relating the chemical constituents of plants to their morphology and evolution (citation from Smith 1976). Chemotaxonomy has since grown in importance; it is a particularly useful tool for plant systematics (Smith 1976), but has also been used in studies of animals (Wright 1974) and microorganisms (Ragan and Chapman 1978). The purpose of bringing the two seemingly divergent disciplines of chemistry and taxonomy together is to find objective characters which can aid in determining the genetic distances between organisms. Smith (1976), Wright (1974), and Ragan and Chapman (1978) have reviewed the extensive literature in this field. Howse and Bradshaw (1980) recently discussed several aspects of social insect chemical systematics.

We have found chemotaxonomy useful also for differentiating fire ant species and castes. In this review, I will discuss the venom alkaloids and cuticular hydrocarbons we have found useful in distinguishing fire ant species and then deal in more detail with how these characters have been used to address a variety of fire ant research problems. Finally, I will show how the use of the alkaloids and hydrocarbons, in conjunction with Dufour's gland components, have been used to demonstrate hybridization between *Solenopsis invicta* and *S. richteri*.

VENOM ALKALOIDS

Chemistry

The poison gland contents of *Solenopsis* species are primarily alkaloids rather than the proteins which are typical of most stinging Hymenoptera. Less than 1% of the venom is proteinaceous (Baer et al. 1979), with the remainder consisting of alkaloids in aqueous suspension. The venom alkaloids are perhaps the most well-known

and studied class of chemicals from fire ants. Their basic structure was first identified correctly by MacConnell et al. (1970) as 2-methyl-6-alkylpiperidine (Fig. 1A, B). Depending upon the species, the methyl and alkyl groups can exist in cis or trans conformations relative to the piperidine ring; also the 6-alkyl group can have several chain lengths and may be saturated or contain a single double bond. For ease of reference, I will use a shorthand notation that specifies the conformation and chain length of the compound and whether it is saturated or unsaturated. For example, cis-2-methyl-6-n-undecylpiperidine is equivalent to cis-C_{11:0} and trans-2-methyl-6-(cis-6-n-pentadecenyl)-piperidine simplifies to trans-C_{15:1}.

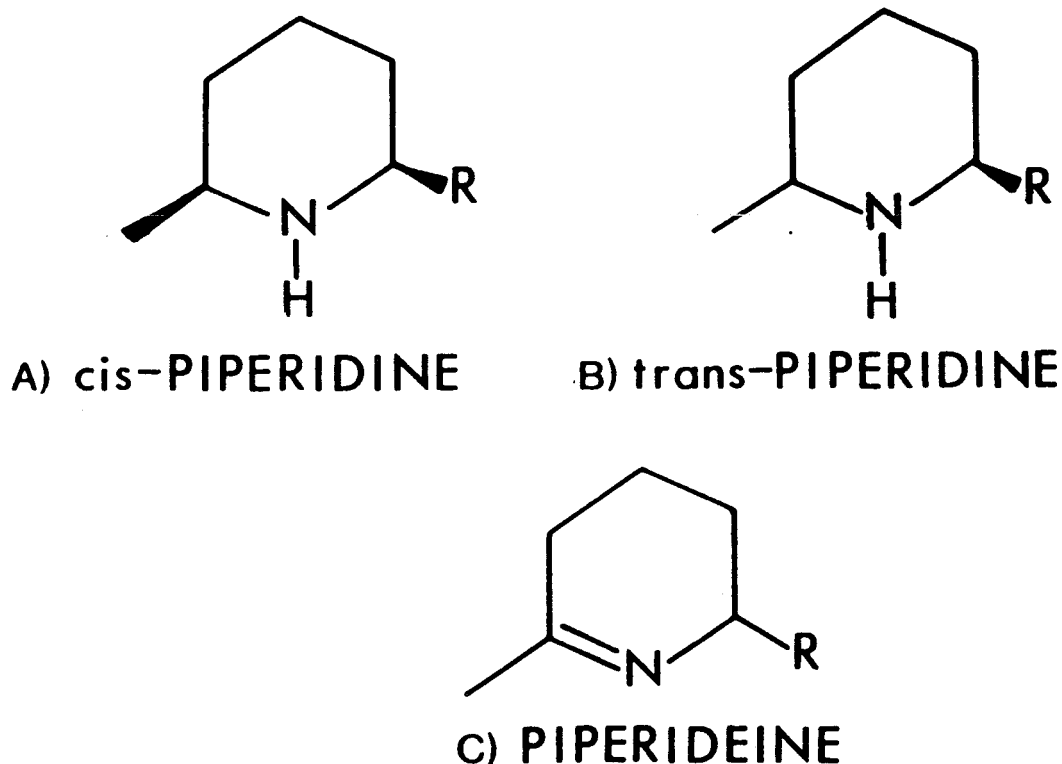


FIGURE 1. General chemical structures of the alkaloids produced by native and imported Solenopsis species in the United States.

Taxonomic Value

Comparative analyses of venom alkaloids from two native Solenopsis species (S. geminata and S. xyloni) and the two imported species (S. invicta and S. richteri) showed alkaloid patterns (Table 1) that clearly differentiated all four species (Brand et al. 1972; MacConnell et al. 1971). It is interesting to note that S. richteri and S. invicta are in the same S. saevissima complex (Buren 1972) and

have qualitatively similar alkaloid patterns although they can be readily distinguished by the absence of C₁₅ alkaloids in S. richteri venom. S. geminata and S. xyloni are in different species complexes (Creighton 1930); and although the cis C_{11:0} alkaloid dominates both chromatograms, S. xyloni is distinctive due to the structurally unique C_{11:0} piperideine (Fig. 1C). In contrast to the species-specific worker venom patterns, alate queens of all four species have similar alkaloid compositions consisting primarily of cis C_{11:0} and trans C_{11:0} alkaloids (Brand et al. 1973).

TABLE 1. Approximate piperidine alkaloid composition for four species of fire ants.^a

Species	Percent piperidine alkaloid in venom					
	Cis C _{11:0}	Trans C _{11:0}	Trans C _{13:1}	Trans C _{13:0}	Trans C _{15:1}	Trans C _{15:0}
<u>S. invicta</u>		minor	20	15	40	20
<u>S. richteri</u>		20	70	10		
<u>S. geminata</u>	60	40				
<u>S. xyloni</u> ^b	70	30				

^a Adapted from Brand et al. 1972 and MacConnell et al. 1971.

^b S. xyloni has a distinctive minor component, C_{11:0} piperideine (Fig. 1).

S. geminata is the only Solenopsis species in the U.S. that has two non-sexual morphological castes, generally referred to as workers and soldiers (soldiers may be a misnomer since their modified large head and mandibles are used primarily for milling seeds). Brand et al. (1973) found that soldiers had a consistently higher ratio of cis to trans C_{11:0} isomers than workers; however, there was considerable variation in this ratio within both castes. A positive correlation between soldier size and the cis to trans ratio led to overlap in the alkaloid isomer ratios of alates and soldiers.

The variation in worker alkaloid ratios in the four major Solenopsis species in the U.S. does not detract from the chemotaxonomic value of venom alkaloids because the differentiating elements are distinguished by their presence or absence. It is not a question of how much.

MacConnell et al. (1976) directly addressed the chemotaxonomic possibilities of venom alkaloids by analyzing the venom composition of 29 populations of 13 New World fire ant species. They concluded that the alkaloids do have taxonomic value but must be used in conjunction with all other taxonomic characters. Analysis of multiple venom samples from the four Solenopsis species in the

U.S. showed almost complete quantitative and qualitative homogeneity. Venom alkaloid samples from the saevissima complex in South America in most cases showed a strong correlation with morphological differences; however, there were a few instances where there were differences in morphology but identical alkaloid patterns. Like any taxonomic character, the venom alkaloids cannot be used alone.

Brand (1978) and Brand et al. (1973) used the differences among venoms of the four fire ant species in the southern U.S. to suggest a scheme for the evolution of venom components that placed the cis C_{11:0} alkaloid closest to the ancestral form. They postulated that this was followed in evolutionary time by a biosynthetic switch to the trans isomer and then to an increase in the alkyl chain length. This model is based on only a few species, but it certainly suggests future research directions.

Minims as a Distinct Caste

The first workers produced by colony-founding queens (called either minims or nanitic workers) have been characterized as more timid (Oster and Wilson 1978) and much smaller (DumPERT and Johnson 1981) than their mature colony counterparts. These general observations led to an investigation of potential biochemical differences between nanitic workers and mature colony workers. In S. geminata, alates and the two worker castes have the same venom alkaloids but in different ratios. The most dramatic caste differences within a species are found in S. invicta female sexuals and workers. The venom of female alates is mainly cis-C_{11:0} and trans-C_{11:0} alkaloids, whereas the four trans C₁₃ and C₁₅ isomers dominate worker venom. Thus, the alkaloid patterns are characteristic of each caste. Surprisingly, minims from newly established colonies had their own distinct pattern consisting of about 94% of the C_{13:1} alkaloid. This is in direct contrast to patterns of both the workers and female alates.

Since minims are the smallest workers produced in a colony, an argument could be made that worker alkaloid patterns vary with worker size and that minims are simply at the extreme end of this variation. Analysis of alkaloids in a range of mature colony worker sizes showed a strong correlation ($r = 0.94$) between worker head-width and the percent C_{13:1} alkaloid. Extrapolation to the minim head-width clearly showed that the new colony minim alkaloid pattern did not fit the mature colony correlation (Vander Meer, unpublished results). These data clearly illustrate that the alkaloid pattern in minims is distinctly different from that of female alates and workers and that minims are not simply small versions of mature colony workers. The behavioral (timid) and morphological (size) factors, coupled with our biochemical studies, led to the conclusion

that minims represent a distinct, although transient, caste.

HYDROCARBONS

Taxonomic Value

Cuticular hydrocarbons have been used as a taxonomic tool in Diptera (Carlson and Service 1980; Carlson and Walsh 1981) and Hymenoptera (Carlson and Bolton 1984). In addition, hydrocarbons have been demonstrated to have a variety of physiological and behavioral roles in insect life (Howard and Blomquist 1982). Nelson et al. (1980) were the first to identify fire ant hydrocarbons, although they did not have chemotaxonomy in mind. They identified the cuticular hydrocarbons of S. invicta and S. richteri as saturated normal, mono, and dimethyl branched hydrocarbons. Although each had the same chromatographic peaks, the relative amounts were so distinctive that they could be diagnostic of the species.

Thompson et al. (1981) identified the five major hydrocarbons in the postpharyngeal gland of S. invicta as the same major components found by Nelson et al. (1980) in cuticular rinses. Further, Vander Meer et al. (1982) found that the postpharyngeal gland of S. invicta female alates and newly-mated queens contain large (15-50 ug) amounts of the species-specific hydrocarbons and that there is a significant quantitative increase shortly after mating. This increase is accompanied by a distinct change in the qualitative pattern. The function of these unusual amounts of hydrocarbons in the postpharyngeal gland is not fully understood.

The characteristic S. invicta pattern of five major components appears to be ubiquitous in S. invicta colonies (Vander Meer et al. 1982) having been found in fat body, larvae, eggs, crop, and even in nest soil. In addition, there appears to be little phenotypic variability since colonies across the southern U.S. maintain the same distinctive qualitative pattern (Vander Meer, unpublished).

The cuticular hydrocarbons of S. geminata and S. xyloni are normal and monounsaturated compounds (Vander Meer, unpublished) with their own distinctive patterns. Therefore, hydrocarbon analysis can be used to differentiate the four major Solenopsis species in the U.S.

Myrmecophile Integration Mechanism

Several myrmecophilous beetles are associated with fire ant colonies. These beetles live in harmony with the ants despite the predaceous habits of the beetles and ants. One of these, Myrmecaphodius excavaticollis, has been found in nests of all four fire ant species. Analysis of cuticular hydrocarbons from beetles collected from a S. richteri colony showed a pattern that was quali-

tatively identical to that of its host's hydrocarbons (Vander Meer and Wojcik 1982). In addition, the beetles contained high molecular weight hydrocarbons not associated with their host. Analysis of beetles that had been removed from the ant host for 14 to 21 days showed that the high molecular weight compounds were innate to the beetle and that the host pattern was probably acquired by the beetles. To test this hypothesis, beetles were removed from S. richteri colonies and after two weeks introduced into S. invicta colonies. After a few days, the surviving beetles were analyzed for hydrocarbons; and, as predicted, they showed a combination of their own innate hydrocarbons and the species-specific hydrocarbon pattern of their new host, S. invicta. Based on these and other experiments, we postulated that the overall mechanism used for integration of M. excavaticollis into its host colonies involved an initial passive defensive behavior (armored exterior and death-feigning) that enabled it to survive long enough to acquire the species odor of its host, as well as the environmental part of the host's colony odor.

Hydrocarbons and Nestmate Recognition

The species-specific hydrocarbons, which account for over 70% of cuticular lipids (Nelson et al. 1980), can be diagnostic of colony odor transfer and may directly contribute to colony odor (Vander Meer and Wojcik 1982). There is not space to discuss nestmate recognition in S. invicta at this time (see Jaffe, Chapter 18, for information on this subject) except to say that multivariate analysis of hydrocarbon patterns indicates that significant differences exist between different colonies and that S. invicta samples can be assigned to their respective colonies based on their cuticular hydrocarbon chromatographic patterns (Vander Meer, unpublished).

CHEMOTAXONOMIC EVIDENCE FOR HYBRIDIZATION

S. invicta and S. richteri are both indigenous to South America. S. richteri is known to occur in central Argentina, Uruguay, and extreme southern Brazil, whereas, S. invicta occurs in southwestern Brazil, Paraguay, and extreme northeastern Argentina (Lofgren et al. 1975). Their distribution in South America and their potential areas of overlap have not been fully defined. S. richteri was the first to arrive in the U.S. in the early 1900s; it was quickly displaced by the more aggressive S. invicta, which arrived in the late 1930s. Presently, S. richteri only occupies enclaves in northern Alabama and Mississippi (see Lofgren, Chapter 4). Although interaction between the two species in South America is not documented, it is certain that under the conditions of their accidental introduction into the U.S., they were forced into direct competition and contact

with each other.

The taxonomy of the S. saevissima complex has had an interesting past and, as we shall see, will have an equally interesting future. Creighton (1930) described 16 forms in the saevissima complex and lumped these into 8 subspecies. Wilson (1952) further consolidated the complex into two species (S. interrupta and S. saevissima) and one subspecies (S. saevissima richteri). Wilson's view (1953, 1958) of the imported fire ant problem was that in South America the red form (S. invicta) and black form (S. richteri) represented two geographically separated races of the same species. When introduced into the U.S., the two forms interbred, producing a population explosion with the red form dominating and replacing the black form except in northeastern Mississippi.

Buren (1972) expanded the saevissima complex to six species. Most importantly, he elevated the red form to species status and named it S. invicta. The main criteria for the classification of S. richteri and S. invicta as separate species were: (1) constant phenetic characters of the two color forms and (2) no evidence for hybridization. However, biochemical evidence based on comparisons of venom alkaloids, Dufour's gland components, and cuticular hydrocarbon patterns demonstrate that hybridization does indeed occur (Vander Meer et al. 1985).

S. richteri is easily differentiated from S. invicta by the absence of C₁₅ alkaloids (Brand et al. 1972). The venom alkaloid pattern of the hybrid contains the C₁₅ alkaloids characteristic of S. invicta, yet its venom pattern is clearly not that of S. invicta either due to the large amount of trans C_{11:0} alkaloid characteristic of S. richteri. Thus, the hybrid exhibits a blend of S. richteri and S. invicta alkaloid patterns.

As mentioned previously, the cuticular hydrocarbon patterns of S. invicta and S. richteri are also diagnostic of the species. Our data show that hybridization is reflected in the hydrocarbon patterns, since the hybrid exhibits a combination of what has been defined as the two pure species patterns.

Comparisons of the Dufour's gland trail pheromone components provides the most dramatic chemical evidence for hybridization. Gas chromatograph traces of extracts of S. invicta and S. richteri Dufour's glands are very different (Barlin et al. 1976). S. richteri has only one major peak (4 ng/WE) designated C-1/C-2 (see Vander Meer, Chapter 17). These components are found in minute quantities in S. invicta (75 pg/WE). We have not been able to detect S. invicta's major Dufour's gland component, Z,E- α -farnesene, in S. richteri Dufour's glands. In the Dufour's gland profile of the hybrid, S. invicta components dominate; however, the comparatively large amount of C-1/C-2 clearly differentiates the hybrid from pure S. invicta.

Behaviorally, a recruitment or point source bioassay demon-

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