Interspecific variation of $\Delta^{1,6}$-piperideines in imported fire ants

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**ABSTRACT**

$\Delta^{1,6}$-Piperideines have been recently reported in the venom of the red imported fire ants, Solenopsis invicta Buren and the black imported fire ants, Solenopsis richteri Forel. However, they have never been quantified in either species. Furthermore, there is no information available about those piperideines in the hybrid imported fire ants (S. invicta × S. richteri). The abundance of six $\Delta^{1,6}$-piperideines was investigated in both species and their hybrid using gas chromatography–mass spectrometer (GC-MS). They include 2-methyl-6-tridecenyl-6-piperideine, 2-methyl-6-pentadecenyl-6-piperideine, 2-methyl-6-heptadecenyl-6-piperideine, 2-methyl-6-tridecyl-6-piperideine, 2-methyl-6-pentadecyl-6-piperideine, and 2-methyl-6-heptadecyl-6-piperideine. S. invicta produced all six $\Delta^{1,6}$-piperideines, whereas, S. richteri did not produce 2-methyl-6-heptadecenyl-6-piperideine and 2-methyl-6-heptadecyl-6-piperideine. The $\Delta^{1,6}$-piperidine profiles of the hybrid was similar to that of S. richteri, except trace amounts of 2-methyl-6-heptadecenyl-6-piperideine and 2-methyl-6-heptadecyl-6-piperideine were found in some of the samples. The ratio of 2-methyl-6-pentadecenyl-6-piperideine to 2-methyl-6-pentadecyl-6-piperideine ($C_{15:1}/C_{15:0}$) was significantly different among two species and their hybrid. In addition to $\Delta^{1,6}$-piperideines, hybrid workers also contained significantly more piperidines than their parent species. This is the first evidence of heterosis of imported fire ants in venom production.

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1. Introduction

Imported fire ants, including the red imported fire ant, Solenopsis invicta Buren, the black imported fire ant, Solenopsis richteri Forel, and the hybrid, Solenopsis invicta × S. richteri, are significant pest ants in the United States. Both S. invicta and S. richteri were introduced into the United States from South America (Buren, 1972). Red imported fire ants have been found in 13 states (Mobley and Redding, 2005); however, the black imported fire ant is confined to a limited area in northern Mississippi, Alabama and Tennessee (Buren et al., 1974; Vander Meer et al., 1985; Oliver et al., 2009). The hybrid was found in Mississippi (Vogt et al., 2003; Streett et al., 2006; Menzel and Nebeker, 2008), Alabama (Diffie et al., 1988), Georgia (Diffie et al., 1988; Gardner et al., 2008) and Tennessee (Gibbons and Simberloff, 2005; Oliver et al., 2009). Imported fire ants are a significant threat to public health due to the workers' venomous stings. The reaction of human beings to the venom can sometimes be life threatening. Since knowledge on chemical constitution is a prerequisite to understanding venom toxicology, the chemistry of the venom of imported fire ants, particularly the red imported fire ant, has been a subject of extensive research.

It was found that 2-methyl-6-alkyl or alkylpiperidines are the major components in the venom of imported fire ants (MacConnell et al., 1970, 1971, 1974, 1976; Brand et al., 1972; Blum et al., 1992). The chemical structures of those piperidine alkaloids have been determined. The alkyl or alkyl side chain on position 6 of the piperidine ring has 11, 13, 15, or 17 carbons. Piperidines with 17-carbon side chains were
found only in S. invicta. For an alkenyl side chain, there is one double bond on the chain and it is always in cis configuration. Since there are two substituents on the piperidine ring, there are two ring configurational isomers for each piperidine alkaloid. Both isomers were found in the venom with trans isomer as the dominant one. The absolute configuration of the trans piperidine alkaloids is always (2R, 6R) while that of the cis is (2R, 6S) (Leclercq et al., 1994). There is a significant difference in the profile of piperidines between S. invicta and S. richteri. The profile of the hybrid was believed to be the mixture of its parent species (Ross et al., 1987).

Deslippe and Guo (2000) investigated the piperidine alkaloids of fire ants in relation to worker size and age. They found the relative abundance of piperidine alkaloid in S. invicta was correlated with worker size and ratios of saturated to unsaturated alkaldoids were positively correlated with worker size. Venom synthesis in S. invicta was found to be limited to early life, and venom dose per sting is modulated (Haigh and Tschinkel, 2003).

Hybrid imported fire ants were found many years after the introduction of its parent species. S. invicta and S. richteri had been treated as subspecific variants until Buren (1972) separated them into two species. One support for such separation was that no hybrid between these two species was found at that time (Buren, 1972). Vander Meer et al. (1985) reported ants collected near the contact zone of two species in Mississippi exhibited intermediate profiles of piperidine alkaloid and cuticular hydrocarbons, confirming the existence of a hybrid between these two species. It is difficult, if not impossible, to distinguish the hybrid from the parent species just based on the morphological characteristics. Vander Meer et al. (1985) developed a method to distinguish the hybrid from its parent species based on piperidine and cuticular hydrocarbon profiles. Two decades later, this method is still playing an important role in identifying hybrid imported fire ants (Vogt et al., 2003; Gibbons and Simberloff, 2005; Streett et al., 2006; Gardner et al., 2008; Menzel and Neebeker, 2008; Oliver et al., 2009).

Piperidine alkaloids have recently been identified from extracts of poison glands of the red imported fire ants (Chen et al., 2009), worker body extractions of the red imported fire ant (Chen et al., 2009; Chen and Fadamiro, 2009a) and the black imported fire ant (Chen and Fadamiro, 2009b). They include 2-methyl-6-alkyl or alkenyl-6-piperideines (Δ16-piperideines) and 2-methyl-2-alkyl or alkenyl-2-piperideines (Δ12-piperideines). Chen et al. (2009) developed a method to separate and purify six Δ16-piperideines from extracts of poison glands and worker body extractions, making it possible to obtain the profile of NaBH4 reduction products of all six Δ16-piperideines. NaBH4 reduction products and high resolution mass spectrometry were used to finalize the elucidation of their chemical structures (Chen et al., 2009).

The discovery of piperideine alkaloids is important in further understanding the toxicological, physiological, and behavioral function of the imported fire ant venom. Since the piperidine profile has been used in chemotaxonomy of imported fire ants, these piperideines may also be valuable in identifying those ant species. However, they have never been quantified in either species. Furthermore, there is no information available about those piperideines in the hybrid imported fire ant. In this study, the absolute and relative abundance of six Δ16-piperideines was investigated in both species and their hybrid using gas chromatography–mass spectrometer (GC–MS). They include 2-methyl-6-tridec-9-enyl-6-piperideine (referred thereafter as C19:1, where 13 is the carbon number of the side chain on the position 6 of the piperideine ring and 1 represents the number of double bond on the chain), 2-methyl-6-tridec-9-enyl-6-piperideine (C19:0), 2-methyl-6-pentadec-10-enyl-6-piperideine (C15:1), 2-methyl-6-pentadec-10-enyl-6-piperideine (C15:0), 2-methyl-6-heptadec-11-enyl-6-piperideine (C17:1), and 2-methyl-6-heptadec-11-enyl-6-piperideine (C17:0). Since no peaks of Δ12-piperideines were detected by the method used in this study, this study focused only on Δ16-piperideines.

2. Materials and methods

2.1. Ants

Red imported fire ant colonies were collected from Sharkey County, Washington County, Lamar County, and Yazoo County, Mississippi; black imported fire ant colonies from Desoto County, Mississippi and Madison County, Tennessee; hybrid imported fire ant colonies from Giles County, Alabama, Grenada County, Mississippi and Madison County, Tennessee. Ant mounds were shoveled and placed in a 19-L plastic bucket. The inside wall of the bucket was then coated with baby powder to prevent ant escape. A water-drip method developed by Banks et al. (1981) was used to separate ants from soil. Method of colony maintenance was the same as that described in Chen (2009). Profiles of cuticular hydrocarbons and venom alkaloids were used to identify species and the hybrid (Ross et al., 1987). Social form of S. invicta was determined using PCR (Valles and Porter, 2003).

2.2. Chemicals

Hexane and acetone (99.9% purity, A.C.S. HPLC grade) was used for extraction and purification of the venom alkaloids and hexacosane was used as internal standard for GC–MS analysis. All were purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Relative abundance of six Δ16-piperideines

Ten monogynic colonies of red imported fire ant, 20 colonies of black imported fire ant, and 10 colonies of hybrid imported fire ant were used. A single sample was prepared for each colony. The method developed by Chen et al. (2009) was used to isolate Δ16-piperideines. This extraction involved three steps: placing 2.5 g of workers in 15 ml hexane in a 50 ml beaker at 20 °C for 10 min; transferring the hexane extract into a 20-ml vial; and concentrating the extract to 200 μl under air flow. All extracts were immediately used for further isolation and purification by gravity column chromatography. A column (1.5 cm × 15.0 cm, Wilmad LabGlass, Buena, NJ) with 3.0 g silica gel (Davison®, Grade 636, pore size 60 &angst; 35–60 mesh, St. Louis, MO) was used. The column was first washed with 8 ml hexane and 200 μl whole body extract was loaded. A hexane/acetone mixture was used as the mobile
phase. The procedure consisted of the following steps: 20/0, 8 ml; 19/1, 4 ml; 18/2, 4 ml; 17/3, 8 ml and then 17/3, 12 ml. Only the eluent of the last step was collected. The collection was concentrated to 200 µl and 2 µl was injected for GC–MS analysis. The relative abundance of each of six Δ¹⁶-piperideines was calculated using the peak area.

2.4. Estimation of absolute abundance of the total Δ¹⁶-piperideines

Because the GC peak of 2-methyl-6-pentadecenyl-6-piperideine (C₁₅:₁) was well separated from those of piperideines in the hexane ant extract without any cleanup, the relative abundance data obtained above can be used to estimate the amount of the total Δ¹⁶-piperideine as long as the total amount of venom alkaloids is known. For example, assuming the relative abundance of C₁₅:₁ (% of total Δ¹⁶-piperideines) is α and its peak area in a particular hexane extraction is A_C₁₅:₁, the total peak area of Δ¹⁶-piperideines equals to (A_C₁₅:₁ × 100)/α. If the amount of total alkaloid (piperideines + piperideines) is N_total, and peak area of total alkaloids is A_total, the amount of total piperideines (N_piperideines) can be estimated using following equation:

\[ N_{\text{piperideines}} = \frac{N_{\text{total}} \times A_{\text{C₁₅:₁}} \times 100}{(\alpha \times A_{\text{total}})} \]

An assumption for this method of estimation is that piperideine alkaloids have similar GC–MS responses as piperideine alkaloids, in term of peak areas. Due to their structure similarity, such estimation is most likely valid. In order to get the total amount of alkaloid (piperidesines + piperideines), a standard curve was developed using purified total alkaloids for each species and hybrid. Total alkaloids were purified from ant hexane extraction using column chromatography. The sample preparation, column and silica gel were the same as that described above for isolating Δ¹⁶-piperideines; however, only 1.0 g silica gel was used. The column was first eluted with 12 ml hexane which washed out all hydrocarbons, and then 40 ml acetone. Only acetone elute which contained venom alkaloids was collected. After the acetone was evaporated under the air flow, the pure venom was weighed. A series of venom alkaloid solutions was then prepared with hexacosane hexane solution (0.75 mg/ml). Hexacosane was used as an internal standard. The ratios of peak area of all venom alkaloids to that of hexacosane were used to develop standard curves. Three colonies from each of 3 sites were used for each species and hybrid. Ten workers were randomly collected from each colony. Workers were killed by being placed in a freezer at –20°C for 5 min. Each worker was placed in a 100 µl insert and ground using a fine glass pipette and 50 µl of hexacosane solution (0.75 mg/ml in hexane) was then added. The extract was subjected to the GC–MS analysis. There was a total of 90 samples for each species. All S. invicta colonies except one were monogyne.

2.5. Gas chromatography–mass spectrometry (GC–MS) analysis

A Varian GC–MS system was used. It consisted of a CP-3800 gas chromatograph and a Saturn 2000 mass selective detector, which were controlled by Mass Spectrometry WorkStation Version 6.41 (Varian, Walnut Creek, CA). A 30 m × 0.25 mm DB-1 capillary column with 0.25 µm film thickness was used (J & W Scientific, Folsom CA). The GC temperature program was as follows: initial temperature was 50°C, held for 1 min increased to 250°C at a rate of 20°C/min, held for 40 min. The split ratio was 1:10, injection temperature was 250°C, transfer line temperature was 200°C. Helium was used as the carrier gas and the flow rate was 1.0 ml/min. The mass spectrometer was operated at 70 eV in the electron impact mode.

2.6. Statistical analysis

Analysis of variance followed by LSD mean comparison at α = 0.05 (PROC GLIMMIX, SAS Institute, 2006) was used to compare relative abundance of each Δ¹⁶-piperideine in each species and hybrid. Since S. richteri did not contain C₁₇:₁ and C₁₇:₀, these two compounds were not included in the analysis for S. richteri. Among species, the total alkaloids, the total Δ¹⁶-piperideines, and the percentage of Δ¹⁶-piperideines in total alkaloids were compared. Comparison among sites was conducted separately for each species and comparison among colonies was conducted separately for each species using pooled data from 3 sites. Since only one polygyne S. invicta colony was involved in this experiment, no comparison among social forms was made.

3. Results

3.1. Relative abundance of six Δ¹⁶-piperideines

Profiles of Δ¹⁶-piperideines in black imported fire ants and hybrid imported fire ants were different to that of red imported fire ants (Figs. 1 and 2). C₁₃:₁, C₁₃:₀, C₁₅:₁ and C₁₅:₀ were found in every sample; however, C₁₇:₁, and C₁₇:₀ were only found in all samples of red imported fire ants and 2 of 10 hybrid imported fire ant samples. In S. invicta, the relative abundance of C₁₅:₀ was significantly higher than any other Δ¹⁶-piperideines (F = 164.61; df = 5, 54; P < 0.0001). C₁₅:₁ was always the most dominant Δ¹⁶-piperideines in S. richteri (F = 722.13; df = 3, 72; P < 0.0001) and hybrid (F = 295.93; df = 5, 54; P < 0.0001) (Fig. 2).

3.2. Absolute abundance of the total Δ¹⁶-piperideines

A significant difference was found in the abundance of total Δ¹⁶-piperideines among species based on both body weight (F = 29.52; df = 2, 267; P < 0.0001) and individual ant (F = 25.86; df = 2, 267; P < 0.0001) (Table 1). The hybrid contained significantly more Δ¹⁶-piperideines than its parent species; however, the difference between S. invicta and S. richteri was not significant. In the hybrid, on average, 3.06% of the total alkaloid was Δ¹⁶-piperideines. The largest percentage of Δ¹⁶-piperideines in the total alkaloids was found to be 15.28% in an hybrid worker from a colony collected from Grenada County, Mississippi (Fig. 3). The abundance of piperidine alkaloids was also significantly different among species and the hybrid, based on both body weight (F = 55.57; df = 2, 267; P < 0.0001) and individual
ant ($F = 48.14; df = 2, 267; P < 0.0001$) (Table 1). Again the hybrid contained significantly more piperidines than its parent species. The difference in piperidine abundance based on ant body weight was significant between S. invicta and S. richteri; however, the difference based the abundance per ant was not significant. A significant difference in percentage of $\Delta^{15}$-piperidines in the total alkaloids was found among species ($F = 7.29; df = 2, 267; P = 0.008$). Hybrids contained significantly higher percentage of $\Delta^{15}$-piperidines in the total alkaloids than its parent species. Between S. invicta and S. richteri, the difference in the percentage of $\Delta^{15}$-piperidines in the total alkaloids was not significant.

In S. invicta, there was a significant difference in the abundance of $\Delta^{15}$-piperidines among sites based on the ant body weight ($F = 4.85; df = 2, 87; P = 0.010$) and individual ant ($F = 4.67; df = 2, 87; P = 0.011$). However, there was no significant difference in the abundance of piperidine alkaloid among sites based on body weight ($F = 2.48; df = 2, 87; P = 0.09$) and individual ant ($F = 0.99; df = 2, 87; P = 0.38$). The difference in percentage of $\Delta^{15}$-piperidines in the total alkaloids was significant among sites ($F = 5.32; df = 2, 87; P = 0.007$). The result of analysis using pooled data from 3 sites showed that there was a significant difference in abundance of $\Delta^{15}$-piperidines among colonies based on the ant body weight ($F = 6.96; df = 8, 81; P < 0.0001$) and individual ant ($F = 6.03; df = 8, 81; P < 0.0001$), in abundance of piperidine alkaloids among colonies based on the ant body weight ($F = 3.87; df = 8, 81; P = 0.0007$) and individual ant ($F = 2.34; df = 8, 81; P < 0.026$), and in percentage of $\Delta^{15}$-piperidines in the total alkaloids ($F = 4.37; df = 8, 81; P = 0.0002$). The difference in ant body weight was not significant among colonies ($F = 1.49; df = 8, 81; P = 0.17$).
Table 1
Absolute and relative abundance of Δ^{15}-piperidines in imported fire ants (mean (SE), n = 90 for each species).

<table>
<thead>
<tr>
<th>Species</th>
<th>Δ^{15}-Piperidines (μg/mg)</th>
<th>Individual ant (μg/ant)</th>
<th>Relative abundance of Δ^{16}-piperidines in the total alkaloids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ^{16}-Piperidines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. invicta</td>
<td>0.17 (0.012) b</td>
<td>0.23 (0.017) b</td>
<td>1.78 (0.12) b</td>
</tr>
<tr>
<td>S. richteri</td>
<td>0.39 (0.047) b</td>
<td>0.71 (0.13) b</td>
<td>2.23 (0.20) b</td>
</tr>
<tr>
<td>S. invicta × S. richteri</td>
<td>1.19 (0.17) a</td>
<td>2.31 (0.35) a</td>
<td>3.06 (0.35) a</td>
</tr>
<tr>
<td></td>
<td>Pideridines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ^{16}-Piperidines</td>
<td>10.16 (0.67) c</td>
<td>13.89 (1.19) b</td>
<td></td>
</tr>
<tr>
<td>S. richteri</td>
<td>17.58 (1.33) b</td>
<td>25.00 (2.10) b</td>
<td></td>
</tr>
<tr>
<td>S. invicta × S. richteri</td>
<td>40.99 (3.43) a</td>
<td>66.49 (0.49) a</td>
<td></td>
</tr>
</tbody>
</table>

Means labeled with different letters are significantly different (α = 0.05).

In S. richteri, there was a significant difference in abundance of Δ^{15}-piperidines among sites based on body weight (F = 15.38; df = 2, 87; P < 0.0001) and individual ant (F = 4.67; df = 2, 87; P = 0.0013). There was also a significant difference in the abundance of piperidine alkaloids among sites based on body weight (F = 3.92; df = 2, 87; P = 0.024) and individual ant (F = 5.83; df = 2, 87; P = 0.004). There was a significant difference in the percentage of Δ^{15}-piperidines in the total alkaloids among sites (F = 18.27; df = 2, 87; P < 0.0001). There was a significant difference in the abundance of Δ^{15}-piperidines among colonies based on the ant body weight (F = 10.75; df = 8, 81; P < 0.0001) and individual ant (F = 4.60; df = 8, 81; P < 0.0001), in the abundance of piperidine alkaloids among colonies based on the ant body weight (F = 11.29; df = 8, 81; P < 0.0001) and individual ant (F = 12.44; df = 8, 81; P < 0.0001), and in the percentage of Δ^{15}-piperidines in the total alkaloids (F = 5.88; df = 8, 81; P < 0.0001). There was no significant difference in the abundance of piperidines among sites based on body weight (F = 19.28; df = 2, 87; P < 0.0001) and individual ant (F = 20.56; df = 2, 87; P < 0.0001). The difference in the percentage of Δ^{15}-piperidines in the total alkaloids among sites was not significance (F = 2.97; df = 2, 87; P = 0.057). There was a significant difference in abundance of Δ^{16}-piperidines among colonies based on the ant body weight (F = 11.73; df = 8, 81; P < 0.0001) and individual ant (F = 10.14; df = 8, 81; P < 0.0001), in the abundance of piperidine alkaloids among colonies based on the ant body weight (F = 13.57; df = 8, 81; P < 0.0001) and individual ant (F = 7.68; df = 8, 81; P < 0.0001), and in the percentage of Δ^{15}-piperidines in the total alkaloids (F = 4.35; df = 8, 81; P = 0.0002). There was no significant difference in ant body weight among colonies (F = 0.75; df = 8, 81; P = 0.65).

There was significant difference in the ratio of C_{15:1}/C_{15:0} among species and hybrid (F = 73.15; df = 2, 36; P < 0.0001) (Table 2). This ratio is the greatest in S. richteri, followed by the hybrid and S. invicta. The ratio of C_{15:1}/C_{13:0} was significantly different among species and the hybrid (F = 9.41; df = 2, 36; P = 0.0005) (Table 3). The hybrid had the greatest ratio, followed by S. richteri and S. invicta.

4. Discussion

This study shows that hybrid ants contain significantly more piperidines and piperidines than its parent species. Since venom alkaloid is used in predation and defense (Haigt and Tschinkel, 2003; Haigt, 2006), an increased amount of venom alkaloids should render hybrid ants an advantage when they compete with their parents and increase their fitness. However, Shoemaker et al. (1996) suggested that hybrid imported fire ants have reduced relative fitness compared to their parent species due to the intrinsic selection. Ross and Robertson (1990) also argued that genomic incompatibility between the parent fire ant species apparently overrides any heterotic effects arising in the regulation of development in highly heterozygous hybrids. Aggression toward heterospecifics can be used as...

![Graph](https://via.placeholder.com/150)

Fig. 3. GC-MS-El total chromatogram (TIC) for hexane extraction of a hybrid worker which had the highest percentage of Δ^{15}-piperidines in its total alkaloids (15.28%) among a total of 90 analyzed workers. Peak assignment: 1: trans-2-methyl-6-undeceyipiperidine; 2: cis-2-methyl-6-tridecyipiperidine; 3: cis-2-methyl-6-tridecyipiperidine; 4: trans-2-methyl-6-tridecyipiperidine; 5: trans-2-methyl-6-tridecyipiperidine; 6: cis-2-methyl-6-pentadecyipiperidine; 7: 2-methyl-6-pentadecyipiperidine (C_{15:1}); 8: cis-2-methyl-6-pentadecyipiperidine; 9: trans-2-methyl-6-pentadecyipiperidine; 10: trans-2-methyl-6-pentadecyipiperidine; 11 and 12: unidentified hydrocarbons.

Table 2
Ratio of 2-methyl-6-pentadecyipiperidine (C_{15:1}) to 2-methyl-6-pentadecyipiperidine (C_{15:0}) in the venom of the imported fire ants.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of colonies</th>
<th>Mean (SE)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. invicta</td>
<td>10</td>
<td>0.74 (0.05) c</td>
<td>0.53</td>
<td>0.92</td>
</tr>
<tr>
<td>S. richteri</td>
<td>20</td>
<td>4.00 (0.22) a</td>
<td>2.68</td>
<td>5.96</td>
</tr>
<tr>
<td>S. invicta × S. richteri</td>
<td>10</td>
<td>1.89 (0.15) b</td>
<td>1.14</td>
<td>2.61</td>
</tr>
</tbody>
</table>

Means labeled with different letters are significantly different (α = 0.05).
Table 3
Ratio of 2-methyl-6-tridecynyl-6-piperidine (C<sub>13:1</sub>) to 2-methyl-6-tridecynyl-6-piperidine (C<sub>13:0</sub>) in the venom of the imported fire ant.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of colonies</th>
<th>Mean (SE)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. invicta</td>
<td>10</td>
<td>0.40 (0.097) c</td>
<td>0.022</td>
<td>0.92</td>
</tr>
<tr>
<td>S. richteri</td>
<td>20</td>
<td>1.97 (0.25) b</td>
<td>0.72</td>
<td>5.05</td>
</tr>
<tr>
<td>S. invicta × S. richteri</td>
<td>10</td>
<td>3.47 (0.87) a</td>
<td>0.06</td>
<td>8.20</td>
</tr>
</tbody>
</table>

Means labeled with different letters are significantly different (α = 0.05).

a measure of fitness among sympatric congenic species (Hölldobler, 1983; Obin and Vander Meer, 1989). Results of behavioral bioassays on inter- and intra-specific aggression of imported fire ants are not consistent. Obin and Vander Meer (1989) found no difference among both species and hybrid in aggression toward inter-specific intruders. The hybrid got the highest score for intra-specific (between colonies) aggression and S. invicta the lowest. They argued that the fire ant hybrid zone is maintained in part by hybrid superiority resulting from increased genetic variability in heterozygotes. In contrast, it was found in a recent study that S. invicta had the lowest aggression threshold, whereas S. richteri was the least aggressive (Fadamiro et al., 2009). If the increased abundance of venom alkaloids in hybrid is not able to compensate the genomic incompatibility, it will be very interesting to see what unique characters in behavior, physiology and morphology, make the hybrid a less successful competitor than S. invicta.

As discussed by Shoemaker et al. (1996), the hybrid can have various recombinant genotypes which may have quite different fitness. This study showed hybrids from different colonies have significantly different abundance in both Δ<sup>10</sup>-piperideine, and piperidine. This may serve as more evidence for a great variation of genotypes in hybrid imported fire ants. Research on the fitness of hybrid should include all genotypes in the hybrid zone. Small sample sizes and a limited collection range may contribute to the discrepancy on the results of previous researches on the fitness of hybrid imported fire ants. It may be necessary to separate hybrids on the basis of genotype rather than to treat them as a single class (Shoemaker et al., 1996). How the abundance of venom alkaloids is tied to different hybrid genotypes and their fitness is another interesting question and its answer may be important in predicting the consequence of the hybridization in imported fire ants.

The difference in Δ<sup>10</sup>-piperidine profile between S. invicta and S. richteri was qualitative, as well as that between S. invicta and the hybrid. However, the difference between S. richteri and the hybrid was quantitative, and the only stable difference was that the hybrid had a larger C<sub>15:1</sub> to C<sub>15:0</sub> ratio. This ratio may be useful in distinguishing the hybrid ants from S. invicta; however, it may not be very reliable in differentiating the hybrid from S. richteri, since the maximum ratio found in the hybrid was very close to the minimum ratio in S. richteri.

*Pseudacteon* phorid flies (Diptera: Phoridae) are specific parasitoids of imported fire ants (Porter, 1998). Four species have been introduced into the United States as biological control agents of imported fire ants (Porter et al., 1995, 2004, Orr et al., 1995; Gilbert, 1996; Gilbert et al., 2008). Chen and Sharma et al. (2009) found that nine venom alkaloids, including two piperidines, C<sub>15:1</sub> and C<sub>15:0</sub>, elicited significant antennal activity in *Pseudacteon tricuspis*. Those compounds are attractants to *P. tricuspis*. They proposed a semiochemicals-mediated host location mechanism for *P. tricuspis*. Since the C<sub>15:1</sub>–C<sub>15:0</sub> ratio is significantly different among imported fire ant species and hybrid, does this ratio play any role in host specificity of the phorid flies? If it does, the preference of phorid flies for a certain C<sub>15:1</sub>–C<sub>15:0</sub> ratio may serve a good indicator of its host preference.

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Conflict of interest

None.

References


