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Research article

Venom alkaloids of monogyne and polygyne forms of the red imported fire ant, *Solenopsis invicta*, in Taiwan

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Abstract. Both monogyne and polygyne colonies of *Solenopsis invicta* now occupy Taiwan. Although venom alkaloids of these ants have been described and synthesized, we here report on a quantitative analysis of the two social forms for the first time. The alkaloids were studied by gas chromatography coupled to mass spectrometry (GC-MS), and six major venom alkaloids were detectable in both types of workers. Both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios in alkaloid venom of monogyne workers were statistically higher than that of polygyne workers, but the sum of proportions of unsaturated alkaloids of polygyne workers was significantly higher than that of monogyne workers, regardless of growth temperature, sampling seasons or geographic location. Results of this study demonstrate that the difference in the proportions of unsaturated alkaloids and the ratios of C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ alkaloids might be a good indicator for differentiating monogyne and polygyne forms of *S. invicta.*

Keywords: Solenopsis invicta, monogyne, polygyne, venom, unsaturated alkaloids.

Introduction

The red imported fire ant, *Solenopsis invicta* (Buren), has a sting apparatus that serves in defense, prey capture and antimicrobial action (Blum et al., 1958; Jouvenaz et al., 1972; Braekman and Daloze, 1996). Its venom contains about 90% to 95% water insoluble alkaloids and a small amount of protein (MacConnell et al., 1971; Baer et al., 1979). The alkaloids, termed solenopsins (MacConnell et al., 1970, 1971), exhibit antibacterial, hemolytic, insecticidal and histamine-releasing properties (Blum et al., 1958; Jouvenaz et al., 1972; Lind, 1982), whereas the four major protein allergens in the worker venom are responsible for anaphylactic reactions (Baer et al., 1979; Hoffman, 1987, 1993; Hoffman et al., 1988). Consequently, stings of red imported fire ants represent a significant human health hazard (Stafford, 1996; Vinson, 1997; Kemp et al., 2000). Yi et al. (2003) indicated that three major NOS (nitric oxide synthase) isoforms (nNOS, eNOS and iNOS) should be inhibited by *S. invicta* venom alkaloids, and isosolenopsin A (*cis*-2-methyl-6-*n*-undecylpiperidine), an alkaloid component of fire ant venom, was synthesized and tested for inhibitory activity against three NOS isoforms. The results showed that activities of nNOS and eNOS isoforms were over 95% inhibited with 1000 μM of isosolenopsin A, whereas the enzyme activity for iNOS was inhibited by only about 20% with the same concentration of isosolenopsin A (Yi et al., 2003). Both solenopsin A (*trans*-2-methyl-6-*n*-undecylpiperidine) and isosolenopsin A cause seizures and depress cardiovascular functions in rats (Howell et al., 2005).

Venom alkaloids of *S. invicta* have been analyzed by gas chromatography and have been found to be composed of *cis*- and *trans*-2-methyl-6-alkyl- (or alkenyl) piperidines (MacConnell et al., 1971, 1976; Brand et al., 1972). Absolute configurations of solenopsins have also established 2R, 6S for the *cis* and 2R, 6R for the *trans* alkaloids (Leclercq et al., 1994). The major constituents of venom in workers of *S. invicta* are five to six piperidine alkaloids (i.e., *trans*-2-methyl-6-*n*-undecylpiperidine (*trans* C_{11}); *trans*-2-methyl-6-(*cis*-4'-*n*-tridecenyl) piperidine (*trans* $C_{13:1}$); *trans*-2-methyl-6-*n*-tridecylpiperidine (*trans* C_{13}); *trans*-2-methyl-6-(*cis*-6'-*n*-pentadecenyl) piperidine (*trans* $C_{15:1}$); *trans*-2-methyl-6-*n*-pentadecylpiperidine (*trans* C_{15}); *trans*-2-methyl-6-(*cis*-8'-*n*-heptadecenyl) piperidine (*trans* $C_{17:1}$))

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(MacConnell et al., 1971; Brand et al., 1972; Vander Meer and Lofgren, 1985; Ross et al., 1987; Deslippe and Guo, 2000). The alkyl or alkenyl side chain of these compounds contain from 11 to 17 carbon atoms. Each alkaloid is designated by an abbreviation. For example, *trans* C_{11} denotes the alkaloid with an 11-carbon side chain *trans* to the ring methyl group; $C_{15:1}$ denotes the alkaloid with one double bond in the 15-carbon side chain, and so on.

The blends of alkaloids in fire ant venoms are species-specific. Brand et al. (1972) analyzed the venom alkaloid compositions in four *Solenopsis* species. They found both qualitative and quantitative chemical differences between the venom of these species. MacConnell et al. (1976) investigated the venom alkaloid compositions of workers from 29 populations of 13 New World fire ants species. They noted that the gas-liquid chromatographic (GLC) pattern of alkaloids may reveal taxonomic characters. However, the results must also be combined with morphological characteristics to distinguish among species of *Solenopsis*. Vander Meer (1986) provided useful taxonomic tools to distinguish fire ant species with venom alkaloids and cuticular hydrocarbons.

S. invicta was first discovered in Taiwan in 2003 (Huang et al., 2004), and both monogyny and polygyny were characterized simultaneously (Chen et al., 2006). In the monogyne form, each colony was identified by the presence of a large physogastric queen, and in the polygyne form, ant colonies were identified by the presence of two or more inseminated queens (Porter, 1992). The polymerase chain reaction (PCR) is also used to determine the social form of *S. invicta* colonies (Valles and Porter, 2003). The monogyne *S. invicta* colonies contain individuals with only the $Gp\text{-}9^B$ allele, while polygyne ants have both $Gp\text{-}9^B$ and $Gp\text{-}9^b$ alleles. Krieger and Ross (2002) also found that *Gp-9* encodes an odorant binding protein that most likely belongs to the OBP family. This product may influence social behavior of fire ants; however, this assumption remained to be verified. Although polymerase chain reaction (PCR) technique provides a precise tool for determining the social form of *S. invicta*, it is time-consuming in practice.

Although venom alkaloids of *S. invicta* have been described and synthesized (MacConnell et al., 1971; Leclercq et al., 1994), they have not been compared quantitatively between the two social forms. The major aim of the present investigation is to provide such a quantitative comparison. Here we show that the discrepancy in unsaturated and saturated alkaloids might serve as a taxonomic character for differentiating the social forms of *S. invicta*.

Materials and methods

Collection of Solenopsis invicta *colonies and determination of social forms*

Colonies of *S. invicta* were excavated from Taipei and Taoyuan Counties, and transported to the laboratory in plastic containers that were painted with Fluon™ to prevent escape of ants. Thirty colonies were collected, half being monogyne and half being polygyne colonies. The monogyne colonies were collected from Linkou Township (Taipei County), Longtan, Sanchih and Sinwu Townships (Taoyuan County) whereas the polygyne colonies were collected from Sansia Township (Taipei County) and Bade, Longtan and Pingjhen Townships (Taoyuan County). The social form of *S. invicta* colonies was determined by PCR, according to protocols by Valles and Porter (2003) with little modifications of the PCR profile. Moreover, the social form of *S. invicta* was further confirmed by the presence of queen number in each colony (Porter, 1992). The queen number in polygyne colonies ranged between 2 and 12. The average number of queens per polygyne colony was 5.

Sampling of venom

Two sampling methods were used.

Method I: capillary collection. Pure venom was collected by capillary action in a 5 µl microcapillary tube from major workers of two social forms of *S. invicta* as described previously (Blum et al., 1958). Venom of three workers per colony was pooled and kept in n-hexane at –20°C for GC-MS analysis. Twelve monogyne and polygyne colonies were sampled, respectively.

Method II: whole body soaking. Venom was also collected by whole body soaking. Twenty-five workers of each colony were placed in a single glass vial containing 1 ml n-hexane, and stored at -20°C. After seven days, the solvent was removed and utilized for GC-MS analysis (Ross et al., 1987). Eight monogyne and polygyne colonies were sampled, respectively.

Effect of temperature on venom alkaloid composition

Three monogyne and polygyne colonies of *S. invicta* were excavated to investigate whether temperature influences the alkaloid content in venom. Each colony was separated from soil by slowly flooding the collection bucket (Jouvenaz et al., 1977); then workers from each colony were divided into four groups. Each group of workers was placed in a Fluon-coated plastic container with lid (21 cm long × 14.5 cm wide × 4.5 cm deep), and maintained at 15 °C, 25 °C, 35 °C and room temperature (27 ± 3 °C), respectively. The test tube which was half-filled with water and stoppered with cotton ball was provided for each group. Workers were fed mealworms (*Tenebrio molitor* larvae) and 10 % (wt:vol) sugar solution in cotton-plugged test tubes. The venom of three workers was collected with a microcapillary tube from each group weekly, and pooled in n-hexane for GC-MS analysis. The venom alkaloids were sampled weekly until ants died.

Effect of season on venom alkaloid composition

To investigate whether season affects venom components, three monogyne and three polygyne colonies of *S. invicta* were excavated in August, October, December 2006 and March 2007 (Summer, Fall and Winter 2006, Spring 2007, respectively) in Taipei and Taoyuan Counties, respectively. Venom was sampled from each colony per season and determined according to Method I.

According to the monthly mean climate data from the Central Weather Bureau of Taiwan (http://www.cwb.gov.tw/), the monthly mean temperature in Taiwan was 29.9 °C in August 2006, 25.6 °C in

October and 18.8 °C in December. In March 2007, the monthly mean temperature was 19.7 °C.

Effect of collection location on venom alkaloid composition

Monogyne and polygyne colonies of *S. invicta* were collected in various locations in Taipei and Taoyuan Counties to investigate whether collection location affects the proportions of unsaturated alkaloids in the venom of workers. In Taipei County, ten colonies were collected from three townships. Twenty colonies were collected from four townships in Taoyuan County. Venom was sampled from each colony and determined according to Method I.

Chemical analyses

To separate and identify the alkaloids of *S. invicta*, we used gas chromatography-mass spectrometry (GC-MS) (Finnigan Focus GC coupled to a Finnigan Focus DSQ mass selective detector). Samples were carried through a DB-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) with helium as the carrier gas flowing at a rate of 1.2 ml/min. The oven was programmed from 150 to 270°C at 15°C/min with a 2 min pre-run hold and a 5 min post-run hold. The injection and detection temperatures were set to 250 and 230 °C, respectively. One μl of each sample was injected into the GC-MS system. Xcalibur software (Thermo Finnigan Co.) was used to integrate the area of chromatographic peaks. The mass spectral base peak at m/z = 98 corresponded to the fragment $C_6H_{12}N^+$ (MacConnell et al., 1971).

Statistical analyses

The peak areas corresponding with the six most abundant alkaloids were integrated, and the proportion of each peak area to the sum of peak areas was calculated. The sums of the proportions of unsaturated alkaloids were compared between the two social forms. The C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios were also compared between social forms. These relationships were analyzed using the two-sample *t*-test and two-way ANOVA, using a significant level of $\alpha = 0.05$.

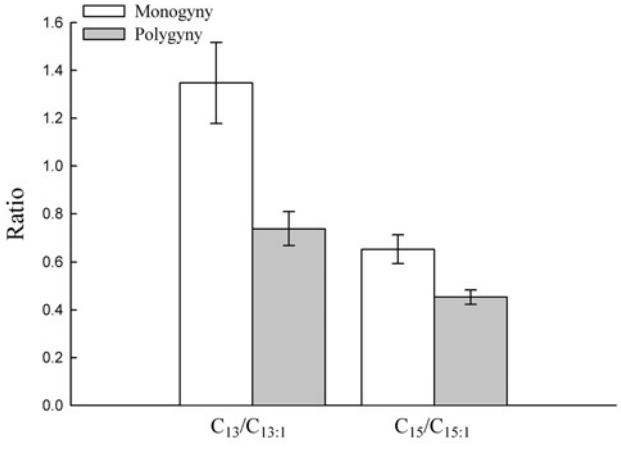

Figure 1. The ratios of peak area (C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$) for workers of monogyne and polygyne forms grown at 15°C for four weeks (for the C_{13} ratio: df = 22, $P = 0.002$; for the C_{15} ratio: df = 22, $P = 0.010$).

Results

The present studies employed GC-MS to analyze the constituents of venom alkaloid sampling from two social forms of *S. invicta* workers by different methods. The GC-MS results of venom alkaloids from two different sampling methods all showed a typical chromatogram contained six major piperidine alkaloids (i.e., *trans* C_{11}, $C_{13:1}$, C_{13}, $C_{15:1}$, C_{15} and $C_{17:1}$) that were detected with retention times between 7 to 11 min in the typical run in Method I. Further, the representative GC-MS chromatogram of venom collected by soaking 25 workers in n-hexane were also detected in Method II. In addition to the six major piperidine alkaloids, cuticular hydrocarbons were also detectable with retention times over 12 min in the GC-MS chromatogram.

Table 1 shows the proportions of unsaturated alkaloids, the C_{13} ratio and the C_{15} ratio in venom of workers from two social forms of *S. invicta* using different sampling methods. Proportions of unsaturated alkaloids in venom from the polygyne form were significantly higher than that from the monogyne form. Moreover, proportions of unsaturated alkaloids in venom obtained by Method II were generally higher than that obtained by Method I.

Since C_{13}, $C_{13:1}$ and C_{15}, $C_{15:1}$ are the most abundant piperidine alkaloids in *S. invicta* venom, we further compared the ratio of C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ in the venom of *S. invicta.* The results showed that both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios in the monogyne form were higher than that of the polygyne form (Table 1).

Table 2 further shows the proportions of unsaturated alkaloids in venom from both social forms of *S. invicta* grown under different temperatures. The proportions of unsaturated alkaloids of the polygyne were significantly higher than that of the monogyne form regardless the growing temperature. The results also showed that polygyne workers incubated at 15°C survived for eight weeks in laboratory environment, whereas, polygyne workers maintained at 35°C began dying after five weeks. Workers of three monogyne colonies grown under four different temperatures died in succession after four weeks, respectively.

Figure 1 shows the C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios of venom for *S. invicta* workers grown at 15°C for four weeks. The results also showed that C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios for monogyne workers (1.35 ± 0.17 and 0.65 ± 0.07, respectively) were significantly higher than those for polygyne workers (0.74 ± 0.06 and 0.45 ± 0.03, respectively) (df = 22, $P = 0.002$ for C_{13}:$C_{13:1}$ and df = 22, $P = 0.010$ for C_{15}:$C_{15:1}$). These results further supported that both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios in monogyne workers were higher than those in polygyne workers.

S. invicta were collected from the field population and subjected to venom alkaloid analysis to determine the seasonal variation in the components of the secreted venom. The results also demonstrated that the proportions of unsaturated alkaloids in polygynes were higher

Table 1. The proportion of unsaturated alkaloids, the C_{13} ratio and the C_{15} ratio in venom of workers from two social forms of *S. invicta* by using different sampling methods.

Sampling Method	Social form	Proportion of unsaturated alkaloids ($C_{13:1}$, $C_{15:1}$ and $C_{17:1}$) Mean ± SE (%) [a]	$C_{13}/C_{13:1}$ Mean ± SE [b]	$C_{15}/C_{15:1}$ Mean ± SE [c]
I	M	51.42 ± 1.64	1.70 ± 0.13	0.71 ± 0.04
	P	58.17 ± 1.83	1.19 ± 0.08	0.59 ± 0.03
II	M	56.25 ± 2.12	1.12 ± 0.15	0.54 ± 0.02
	P	66.13 ± 1.85	0.63 ± 0.09	0.42 ± 0.04

I: capillary collection; II: whole body soaking. M: monogyny; P: polygyny.
[a] Mean proportion of unsaturated alkaloids in worker's venom. It differed significantly between the monogyne and polygyne forms (method I: df = 22; $P = 0.012$; method II: df = 14; $P = 0.0034$) by using two-sample *t*-test.
[b] Mean C_{13} ratio. It differed significantly between the monogyne and polygyne forms (method I: df = 34; $P = 0.003$; method II: df = 12; $P = 0.016$) by using two-sample *t*-test.
[c] Mean C_{15} ratio. It differed significantly between the monogyne and polygyne forms (method I: df = 34; $P = 0.022$; method II: df = 12; $P = 0.019$) by using two-sample *t*-test.

than that in monogynes ($F_{1,\ 16} = 8.853$; $P = 0.009$, ANOVA), but not with season ($F_{3,\ 16} = 0.403$; $P = 0.753$) (Fig. 2).

Table 3 shows the comparison of venom alkaloids of *S. invicta* collected from different locations. In general, the proportions of unsaturated alkaloids in polygynes were higher than that in monogynes. In contrast, both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios in monogyne workers were higher than those in polygyne workers, with the exception of C_{15}:$C_{15:1}$ ratio for polygyne colonies collected from Pingjhen.

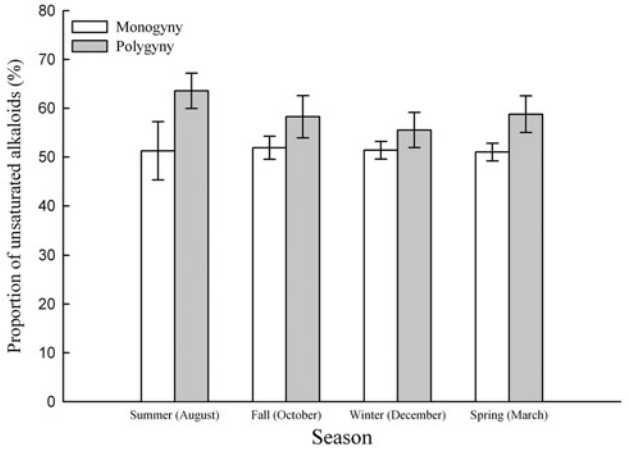

Figure 2. The seasonal variation in proportion of unsaturated alkaloids of venom between monogyne and polygyne forms of *S. invicta* ($F_{1,16} = 8.853$; $P = 0.009$, ANOVA).

Discussion

The venom alkaloids of fire ants have been chemically analyzed. Brand et al. (1972) revealed that the venom of workers of *S. invicta* and *S. richteri* contains various amounts of 2, 6-disubstituted piperidines. Gas chromatographic analyses were performed to distinguish between *S. invicta* and *S. richteri* and their hybrid by Vander Meer and Lofgren (1985). They verified that the venom in *S. invicta* contains abundant C_{15} alkaloids while the venom in *S. richteri* does not. Deslippe and Guo (2000) concluded that intermediately aged workers of *S. invicta* produced more venom than did young and old workers. They also found that the ratios of saturated and unsaturated C_{13} and C_{15} alkaloids differed significantly between minor and major workers. Moreover, Haight and Tschinkel (2003) confirmed that the venom dose varied significantly with worker age and season.

The aforementioned studies raise the question of whether the composition of venom alkaloids differs between monogyne and polygyne forms of *S. invicta.* Much information on the identification of the two social forms of *S. invicta* is available by the locus *Gp-9.* Keller and Ross (1998) defined *Gp-9* as a green beard gene that causes a phenotypic effect. In 2002, Krieger and Ross further characterized that the gene *Gp-9* encodes an odorant binding protein. This product may affect a worker's ability to recognize queens and other individuals. Nevertheless, the biological significance of the *Gp-9*

Table 2. Effect of growing temperature on the proportion of unsaturated alkaloids of workers from two social forms of *S. invicta.*

Temperature	Social form	Proportion of unsaturated alkaloids ($C_{13:1}$, $C_{15:1}$ and $C_{17:1}$) Mean ± SE (%)	df	*P*
15 °C	M	53.92 ± 2.49	22	0.004
	P	63.33 ± 1.50		
25 °C	M	51.48 ± 3.05	10	0.024
	P	62.07 ± 1.92		
35 °C	M	51.89 ± 4.43	8	0.032
	P	65.55 ± 2.89		
Room Temp.	M	52.33 ± 3.57	12	0.007
	P	64.96 ± 1.53		

M: monogyny; P: polygyny.

Table 3. Monogyne and polygyne colonies of *S. invicta* collected from different locations and the proportion of unsaturated alkaloids in venom of workers.

Location	Social form	Colony number	Proportion of unsaturated alkaloids ($C_{13:1}$, $C_{15:1}$ and $C_{17:1}$) Mean ± SE (%) [a]	$C_{13}/C_{13:1}$ Mean ± SE [b]	$C_{15}/C_{15:1}$ Mean ± SE [c]
Sinwu	M	4	51.67 ± 1.50	1.56 ± 0.09	0.71 ± 0.04
Sanchih	M	2	50.50 ± 2.50	1.99 ± 0.14	0.74 ± 0.14
Linkou	M	3	51.33 ± 5.93	1.56 ± 0.42	0.72 ± 0.17
Longtan	M	6	51.33 ± 2.03	1.65 ± 0.19	0.67 ± 0.06
Longtan	P	3	58.26 ± 4.32	1.15 ± 0.32	0.57 ± 0.07
Sansia	P	5	57.58 ± 1.66	1.17 ± 0.13	0.60 ± 0.04
Pingjhen	P	4	52.95 ± 0.52	1.43 ± 0.05	0.69 ± 0.02
Bade	P	3	64.00 ± 3.61	0.72 ± 0.15	0.44 ± 0.06

Colony number: three ants of each colony were analysed. M: monogyny; P: polygyny.
[a] Mean proportion of unsaturated alkaloids in worker's venom. It differed significantly between the monogyne and polygyne forms (df = 28; $P = 0.002$) by using two-sample *t*-test.
[b] Mean C_{13} ratio. It differed significantly between the monogyne and polygyne forms (df = 40; $P = 0.001$) by using two-sample *t*-test.
[c] Mean C_{15} ratio. It differed significantly between the monogyne and polygyne forms (df = 40; $P = 0.004$) by using two-sample *t*-test.

difference between two social forms of *S. invicta* remains unclear.

The present study reveals that the proportions of unsaturated alkaloids were higher in polygyne than monogyne workers. Further, the sampling methods had minor influence on the proportions of unsaturated alkaloids. By using Method II, the proportions of unsaturated alkaloids were higher than pure venom that obtained from workers using the milking method. Lin (2006) investigated the differential cuticular chemical profiles between monogyne and polygyne workers of *S. invicta.* He found that the cuticular chemicals on workers from monogyne and polygyne were mixtures of hydrocarbons, alkaloids and other unknown chemical components. In addition, the amount of the $C_{13:1}$ and $C_{15:1}$ alkaloids from both social forms were higher than other alkaloids. A possible explanation for the higher proportions by using Method II is that there were more unsaturated alkaloids on the cuticle of workers.

Early studies showed that the venom of *S. invicta* contains five major alkaloids, all in the *trans* configuration, with a trace amount of the *cis* form (Brand et al., 1972). A more recent investigation demonstrated that six main alkaloids were detected in major workers (Deslippe and Guo, 2000). Our study confirms the detection of six alkaloids in major worker's venom. Brand et al. (1972) demonstrated that *trans* $C_{13:1}$ and *trans* C_{13} are present in the venom in about equal amounts, but they also found that sometimes the *trans* $C_{13:1}$ content exceeded the *trans* C_{13} content. Deslippe and Guo (2000) determined that the C_{13}:$C_{13:1}$ ratio of major workers is approximately one. In our study, the amount of *trans* C_{13} significantly exceeded that of *trans* $C_{13:1}$ in the monogyne form. We also demonstrate that the amount of *trans* C_{15} is less than that of *trans* $C_{15:1}$ in the venom, as reported by MacConnell et al. (1976) and Deslippe and Guo (2000).

The recent invasion of polygyne and monogyne forms of *S. invicta* into Taiwan substantially impacts local residents. To understand better the aggressive and attack behavior, our study systematically investigated the components of venom for red imported fire ants. The results showed significant difference for the ratios of saturated to unsaturated alkaloids between monogyne and polygyne colonies of *S. invicta* venom. Brand et al. (1973a) demonstrated that the ratio of *cis* C_{11} to *trans* C_{11} was related to the length of the head. Further, the ratios of saturated to unsaturated C_{13} and C_{15} alkaloids were positively correlated with worker size and increased with worker age (Deslippe and Guo, 2000). Both the ratio of saturated to unsaturated C_{13} and C_{15} alkaloids differed significantly between monogyny and polygyny when venom was obtained by milking method (Method I). When another sampling method was used (Method II), the ratios of saturated to unsaturated C_{13} and C_{15} alkaloids also differed significantly between monogyne and polygyne forms. Our studies also showed that the ratio of C_{13}:$C_{13:1}$, in spite of sampling methods, always exceeded that of C_{15}:$C_{15:1}$, as reported by Deslippe and Guo (2000). Moreover, the current results also revealed that the ratios of both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ in monogynes are higher than that in polygynes. When fire ants were incubated at 15°C for one to two months, the ratios of saturated to unsaturated C_{13} and C_{15} alkaloids still differed significantly between monogyne and polygyne forms. These results suggest that the ratios of saturated and unsaturated alkaloids might be a stable characteristic for both social forms of *S. invicta* and might be useful to distinguish monogyne and polygyne forms of *S. invicta.* The ratios of saturated to unsaturated alkaloids differ between the two social form is probably a reflection of a founding event when the polygyne form evolved from the monogyne form. However, further investigations are required to verify this assumption.

The red imported fire ant has expanded its distribution from South America (tropical area) to North America, Australia and some area of Asia (subtropical area). Since various temperature regimes are involved, we incubated *S. invicta* at different temperatures to determine whether the composition of the venom of workers varies with the temperature or the season. Although growing fire ants at a constant temperature condition is unrealistic, this investigation established that the proportion of unsaturated alkaloids averaged 50–55% in workers of the monogyne form and 61–66% in workers of the polygyne form. Though low temperature is the least favorable for fire ant, workers of polygyne colonies sustained longer at 15°C compared to other colonies died successively after laboratory incubation at other temperatures for four weeks. The reason is currently unknown; however, it is possible that the structure of colony and the humidity in laboratory are not the same as that of in the field. Furthermore, limited diet of just mealworms and sugar water may be also the cause for the early mortality of colonies. When workers grew at 15°C for eight weeks, the proportions of unsaturated alkaloids increased from 61% to 72% (data not shown). This result suggests that the proportions of unsaturated alkaloids may increase with rearing time. However, more information is required to verify this hypothesis. The present results also revealed that proportions of unsaturated alkaloids varied significantly between monogyny and polygyny, but not with season.

The proportions of unsaturated alkaloids differed significantly between the monogyne and polygyne forms, even though they were collected from various locations. Conversely, both C_{13}:$C_{13:1}$ and C_{15}:$C_{15:1}$ ratios in monogyne forms were also higher than that in polygyne forms regardless of geographic location. The C_{15}:$C_{15:1}$ ratio of polygyne colonies collected from Pingjhen is an exception and will be the focus for the future investigation. These results further suggest that higher proportions of unsaturated alkaloids in polygyne workers are universal, regardless of growth temperature, sampling season and geographic location. Interestingly, we also noticed that the $C_{17:1}$ alkaloid content in the venom of monogynes significantly exceeded that of polygynes (data not shown) and as the amount of $C_{13:1}$ decreased, the amount of $C_{17:1}$ increased. Nevertheless, the $C_{17:1}$ alkaloid in venom is a minor component. So the amount of $C_{17:1}$ alkaloid would not influence the sum of proportions of unsaturated alkaloids greatly. Jouvenaz et al. (1972) tested the effect of fire ant venom alkaloids on gram-positive bacteria, and found that alkaloids (C_{11}, C_{13} and C_{15}) were toxic to bacteria, and the toxicity tended to decrease from C_{11} to C_{15}. However, Brand et al. (1973b) hypothesized that the venom of descendants of *Solenopsis* species synthesize *trans* piperidines with a long side-chain, which may be responsible for greater toxicity against predators. Thus, more information is required to establish whether the quantity of unsaturated alkaloids influences their toxicities or behavior. We speculate that the difference between the venom compositions of monogynes and polygynes might be associated with important biological functions.

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