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Fatty Acids Differ Significantly in Castes of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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Abstract

Identification and quantitation of fatty acids (FAs) in nymphs, alates, workers, presoldiers, and soldiers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were determined by gas chromatography–mass spectrometry, showing quantitative and qualitative differences among groups. Total FAs content of nymphs and alate females was about 1.5-fold higher than alate males, about 2-fold higher than workers, 6-fold higher than presoldiers, and 12-fold higher than soldiers. Overall differences in total FAs content were due to oleic acid (C18:1), stearic acid (C18:0), linoleic acid (C18:2), and palmitic acid (C16:0). Soldiers contained two unique FAs among the castes—lignoceric (C24:0) and hexacosanoic acid (C26:0). Nymphs had the highest ratio between triacylglycerols and phospholipids probably for energy storage in alate development. Four branched FAs—13-methyl myristic, 14-methyl pentadecenoic, 15-methyl palmitic, and 14-methyl palmitic—and three odd-numbered FAs—pentadecanoic (C15:0), heptadecanoic (C17:0), and heptadecenoic (C17:1)—were found in nymphs, alates, workers, presoldiers, and soldiers. Interestingly, all FAs were distributed in different percentages both in triglycerides and phospholipids of the different developmental stages and castes, indicating a function both for energy storage and membrane components. Five different 2-hydroxy FAs—2-OH C16:0, 2-OH C18:0, 2-OH C20:0, 2-OH C22:0, and 2-OH C24:0—were identified, the latter only in soldiers. Total 2-hydroxy FAs content in soldiers was significantly higher than that in other groups (6.01–7.9-fold vs. presoldiers and 41.7–132.6-fold vs. nymphs, alates, and workers), and the quantity in presoldiers was significantly higher than in nymphs, alates, and workers, with no difference among nymphs, alates, and workers.

Key words: long-chain fatty acids, branched fatty acids, odd-numbered fatty acids, GC-MS

Fatty acids are physiologically important molecules, having diverse functions that include membrane structure, energy storage, pathogen resistance (Stanley-Samuelson 1994), predation avoidance (Nilsson et al. 2004), insect repellency (Henderson et al. 1991, 1993), and are involved in various signaling pathways (Nishizuka 1992, Glatz et al. 1995). Although fatty acids have been characterized, quantified, and analyzed for their physiological functions in several insect species, few studies have been performed in termites. A study of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, by Mauldin et al. (1972) reported that, predictably, fatty acid content changed in partially or completely defaunated workers when compared to normally fed workers. In our previous studies, lignoceric acid, hexacosanoic acid, and ceramides were major components of frontal gland secretion of the Formosan termite soldier (Chen et al. 1999, Ohta et al. 2007). Ohta et al. (2007) characterized three different ceramides from the frontal gland secretion, finding two with C18:0, C20:0, and C22:0 fatty acids and a third with 2-hydroxy C18:0, C20:0, C22:0 fatty acids, as well as unique sphingosines. In *Reticulitermes flavipes* (Kollar), Carter et al. (1972) reported that fatty acid content in workers was different when the termites fed on decayed compared to undecayed wood. We are not aware of any published study of fatty acid composition differences reported in nymphs, alates including males and females, workers, presoldiers, or soldiers of any termite species. It is well-known that *C. formosanus* is one of the most destructive termite species and causes a tremendous amount of economic losses worldwide each year (Zhu et al. 2001). *C. formosanus* treatment and damage costs are at least US$3 billion annually in the United States (Su 2002). The break-down of levees and floodwalls during Hurricane Katrina in New Orleans in 2005 might have resulted at least in part from termite infestations (Henderson 2008). In the present study, we characterized and quantified fatty acid content in nymphs, alates, workers, presoldiers, soldiers, and alates of *C. formosanus*.

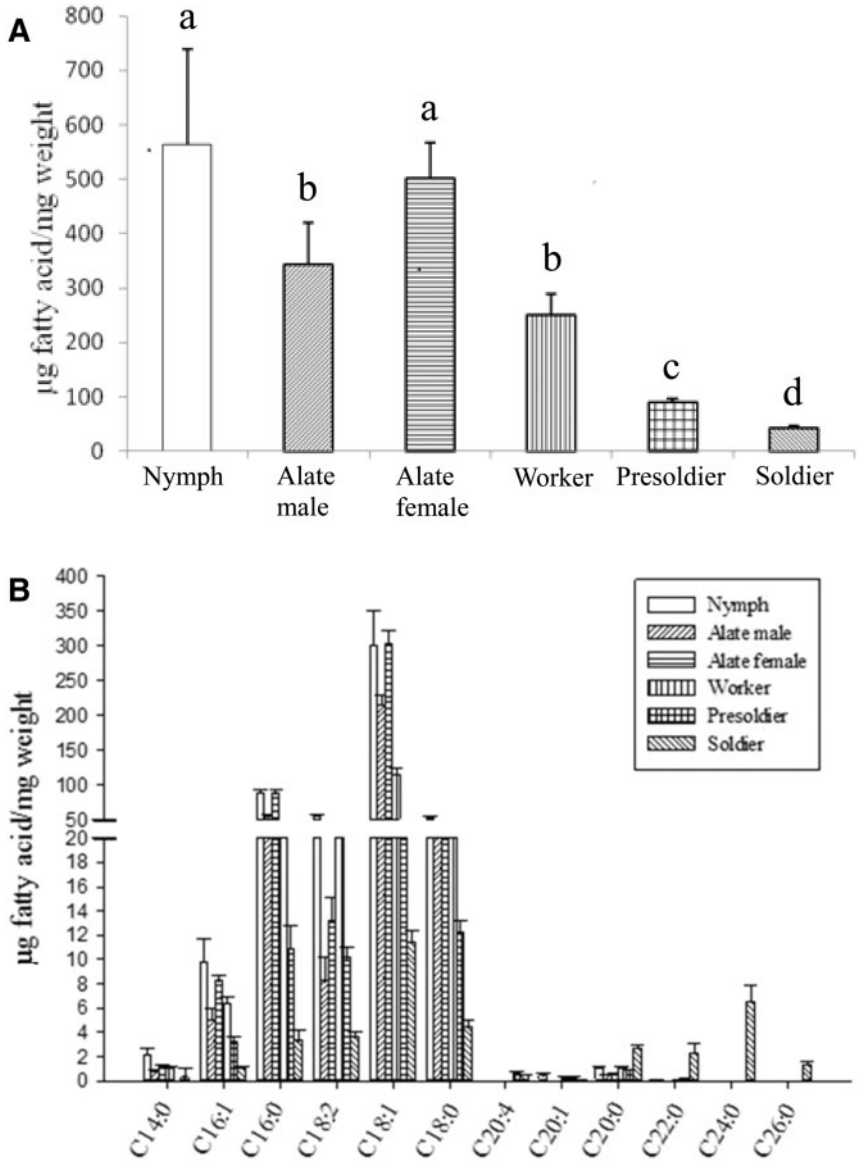

Fig. 1. Fatty acid levels in the Formosan subterranean termite, *C. formosanus*. (**A**) Total fatty acid content in nymphs, alates, workers, presoldiers, and soldiers. (**B**) Fatty acid profiles (excluding branched and odd-numbered fatty acids) of nymph, worker, presoldier, and soldier. Means with the different letters are significantly different ($P < 0.05$). Data are presented as mean ± standard deviation.

Materials and Methods

Chemicals

Fatty acid methyl ester (FAME) standard (GLC 403), 10-heptadecenoic acid (C17:1), and tricosanoic acid were purchased from NuCheck Prep, Inc., Elysian, MN. 13-Methylmyristic, 14-methylhexadecanoic, pentadecanoic, heptadecanoic acids, and bis-trimethylsilyltrifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). 2-Hydroxy fatty acid methyl ester mixtures and 2-hydroxytricosanoic acid were purchased from Matreya LLC. (Pleasant Gap, PA). HPLC grade chloroform, methanol, hexane, ethyl ether, and water were purchased from Fisher Scientific (Houston, TX).

Experimental Insects

A termite colony was collected from sites adjacent to a floodwall, near the French Quarter, New Orleans, on March 20, 2009. Alates were collected from the Parker Termite Rearing Facility, Louisiana State University Agricultural Center, by light traps in May 2009.

Sampling

One individual was used per sample for nymphs, alates, workers, presoldiers, and soldiers ($n = 6$; for presoldier $n = 5$). Male and female alates were separated under a dissecting microscope (Meiji Techno Co. LTD. Tokyo, Japan). All samples were weighed and kept at −20°C until use.

Lipid Extraction

Termite samples were homogenized by hand with a glass/glass homogenizer in 3 ml of methanol: chloroform (2:1 v/v) solution, and the lipids were extracted according to Bligh and Dyer (1959). In all, 50–500 μg of tricosanoic acid (C23:0, 1 μg/μl of

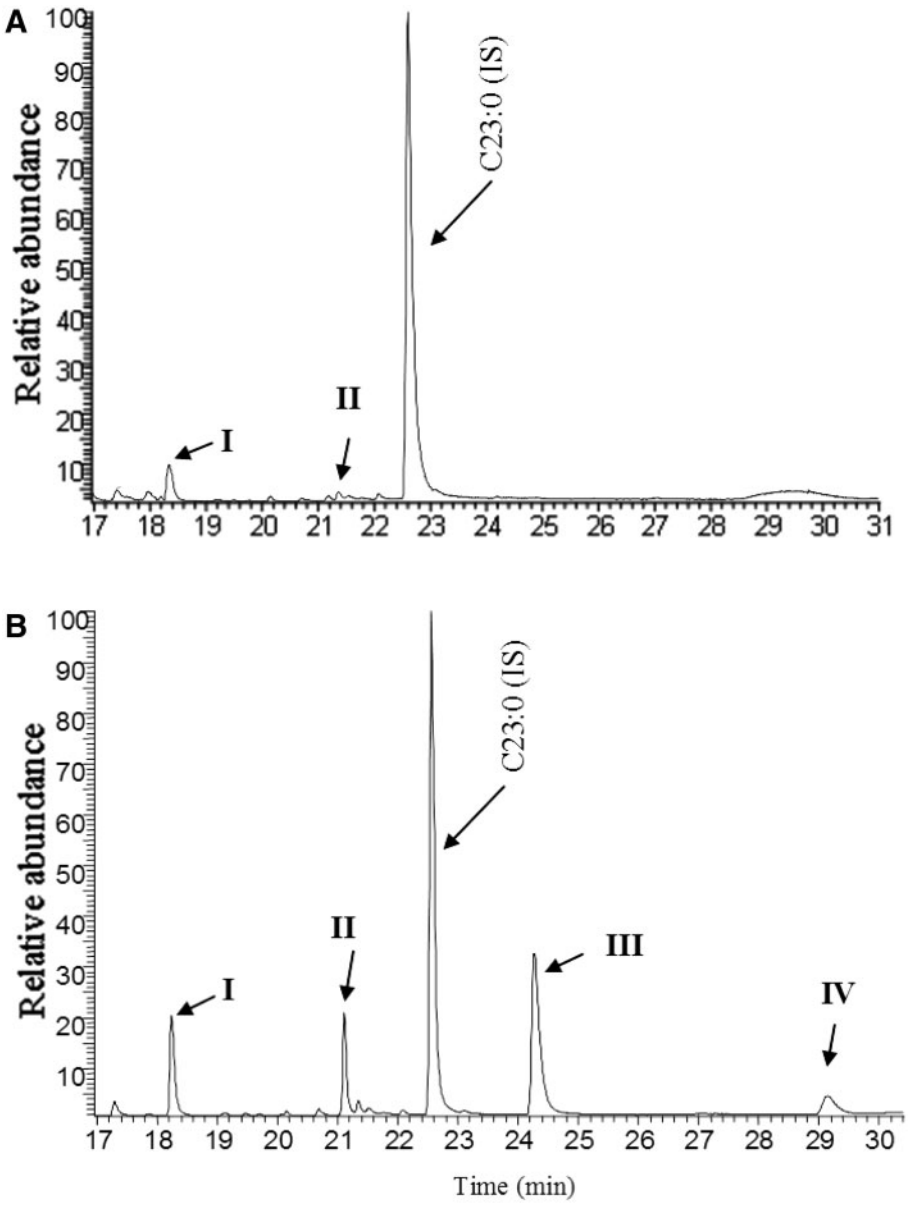

Fig. 2. Late eluting components of the total ion chromatography of workers and soldiers of the Formosan subterranean termite, *C. formosanus*. (**A**) Workers and (**B**) Soldiers. Two peaks representing C24:0 (Peak III, retention time at 24.20 min) and C26:0 (Peak IV, retention time at 29.13 min) occurred in soldiers. Peak I was C20:0 and peak II was C22:0, which were found in all developmental stages and castes (those from nymphs, alates, and presoldiers were not shown since they were the same proportions as in workers). IS, internal standard.

chloroform) based on the body weights (50 µg for small workers, 500 µg for larger members so that internal standards would be in the same order of magnitude as the unknowns) were added to each sample as an internal standard. Lipid extracts were stored in chloroform containing 0.05% (w/v) butylated hydroxytoluene (BHT) at −20°C.

Separation of Lipids From the Formosan Termite by Thin Layer Chromatography (TLC)

To determine the energy storage levels, we compared the ratio between triacylglycerols (TAG) and phospholipids (PL); the total lipid extracts from nymphs, alates, workers, presoldiers, and soldiers ($n = 3$) were separated by TLC on normal phase silica gel plates (10 by 20 cm^2; 250 microns thick, Analtech, Newark Delaware). TLC separation followed the method of Chen et al. (2005 and 2007). Briefly, plates were prewashed in chromatography solvent (hexane: ether: acetic acid; 60:40:1, v/v/v) in a TLC tank lined with filter paper to help saturate the tank atmosphere with solvent vapor. The plate was dried and spotted with samples and standards including monoolein, diolein, triolein, cholesterol oleate, and a phospholipid standard mixture. The standards were dissolved in chloroform. Fifty µg of each standard and the extract volume equivalent to 4 mg body weight of each sample were applied to the TLC plate, which was developed in hexane: ether: acetic acid (60:40:1, v/v). A duplicate TLC plate was developed, visualized with phospholipid fractions and photographed with a Sony Digital Camera (Fig. 6A).

The phospholipid fractions were collected to glass test tubes by razor blade after the plate was placed in a TLC tank containing iodine crystals and allowed to develop for 30–40 min. The plate was held

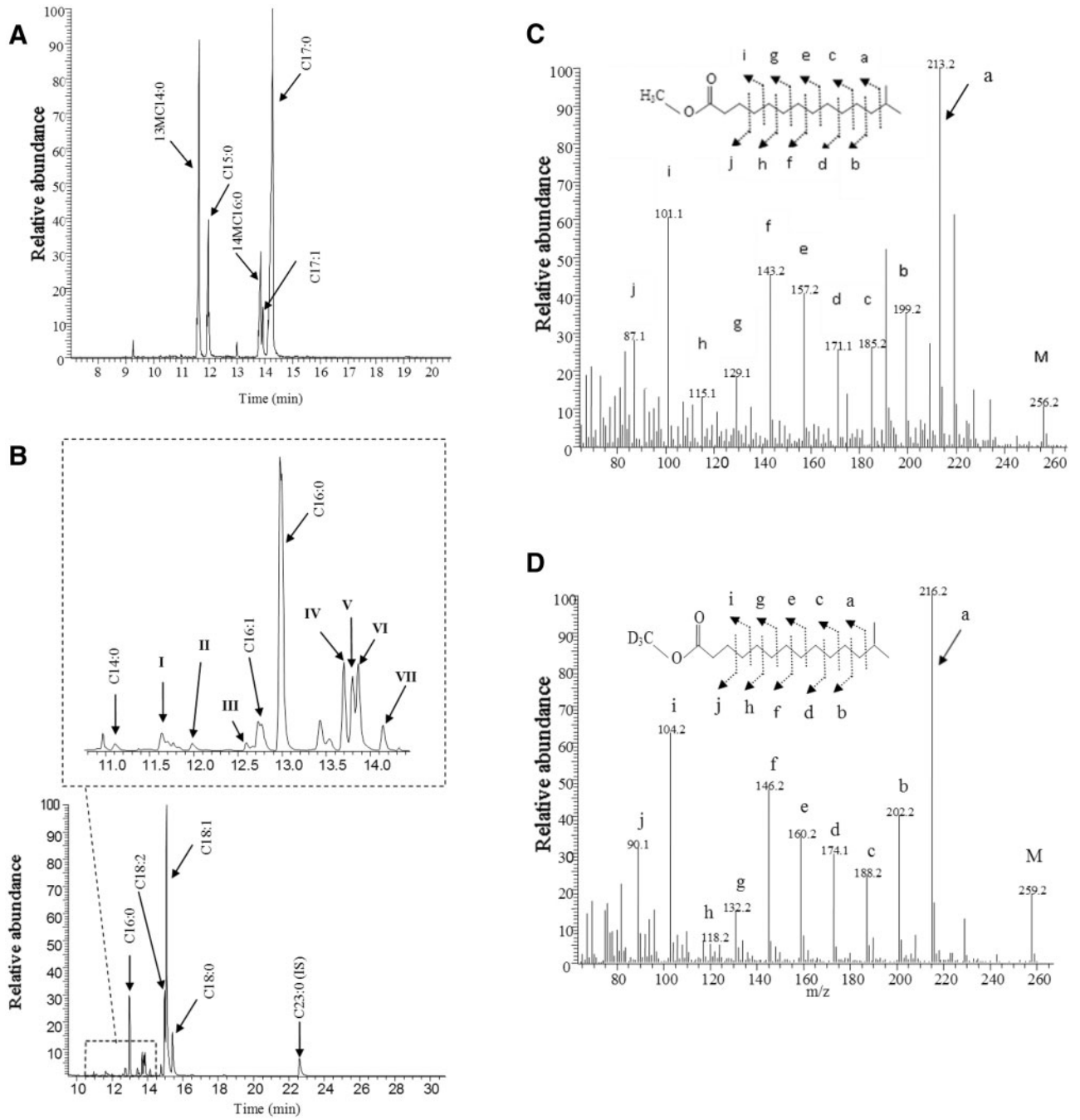

Fig. 3. Identification of branched and odd-numbered fatty acids in *C. formosanus*. (**A**) Standard mixture including 13-methyl C14:0, C15:0, 1-methyl C16:0, C17:1, and C17:0. (**B**) Total ion chromatography of nymph fatty acids. Expanded area shows partial total ion chromatography. (**C**) Ion spectrum of 13-methyl C14:0 methyl ester. (**D**) Ion spectrum of 13-methyl C14:0 methyl-d_3 ester. (**E**) Ion spectrum of 14-methyl C15:0 methyl ester. (**F**) Ion spectrum of 14-methyl C15:0 methyl-d_3 ester. (**G**) Ion spectrum of 15-methyl C16:0 methyl ester. (**H**) Ion spectrum of 15-methyl C16:0 methyl-d_3 ester. After comparison of ion spectra and retention time with the standards, peak I at 11.65 min was identified as 13-methyl myristic acid; peak III at 12.58 min, peak IV at 13.70 min, and peak V at 13.80 min were identified as 14-methyl pentadeconoic acid, 15-methyl palmitic acid, and 14-methyl palmitic acid, respectively. Peak II at 11.95 min, peak VI at 13.89 min, and peak VII at 14.16 min were identified as C15:0, C17:1, and C17:0, respectively.

under a hood and the spots were marked with pencil before iodine vaporized. Each spot was collected to a centrifuge tube by a razor blade after iodine vaporized. These thin layer fractions were extracted with 2 ml of ethyl ether followed by two additional extractions with 1 ml of ethyl ether each time after addition of 50 µg of tricosanoic acid (C23:0; 1 µg/µl) as an internal standard. The supernatant fractions were combined, dried, and fatty acid methyl esters (FAMEs) were prepared as described in the next section. Phospholipids were extracted after addition of 50 µg of tricosanoic acid (C23:0; 1 µg/µl) as an internal standard. Three milliliters of chloroform:methanol:acetic acid:water (50:39:1:10, v/v) were added to the tube, vortexed, and then centrifuged at low speed. The supernatant was removed and the gel extracted with the same solvent. The supernatant fractions were combined and 4 M NH_4OH (1 ml/ 3 ml solvent) added. The tube was vortexed, centrifuged as above, and the chloroform phase was removed. One milliliter of chloroform was added, to re-extract the

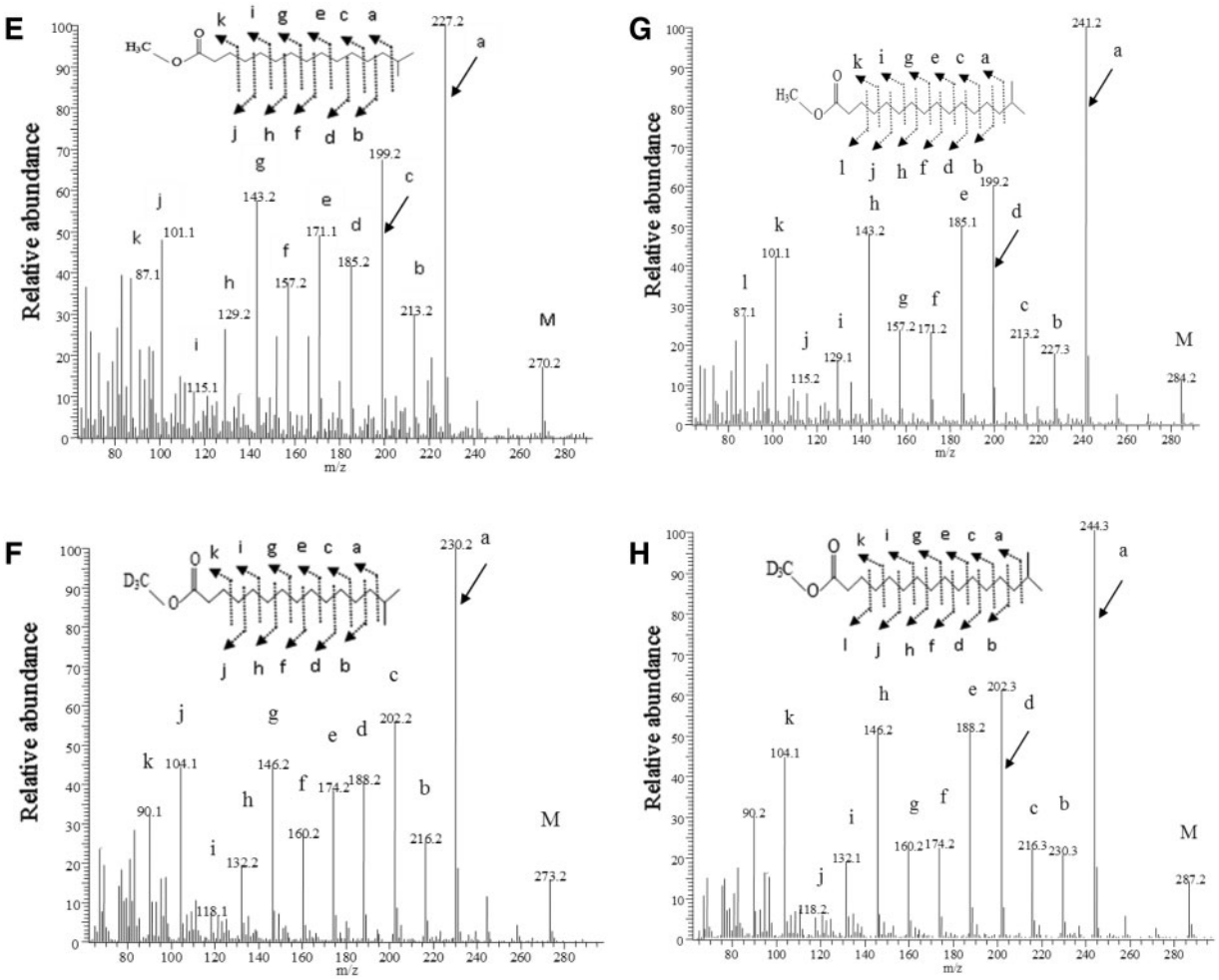

Fig. 3. Continued

silica. The solvent was dried under nitrogen and fatty acid content was determined as described below. Estimates of storage lipid levels in the different developmental stages and castes were calculated as the ratio of TAGs to PLs obtained from extraction.

Fatty Acid Analysis

Methyl esters of fatty acids were prepared and analyzed based on the method of Chen et al. (2005). Fatty acid methyl-d_3 esters were purified and isolated according to Shipley et al. (1993). For determination of methyl branch position, methyl esterification of fatty acids was carried out with addition of 100 µl 1% HCl-methyl-d_3 alcohol, prepared from methyl-d_3 alcohol bubbled with hydrogen chloride gas, to the lipid extracts and held at 100°C until the solvent vaporized. FAMEs and fatty acid methyl-d_3 esters were analyzed by gas chromatography–mass spectrometry (GC-MS) on a Trace gas chromatography (Thermo Quest Inc. Schaumburg, IL), using a DB-5 column 30 m by 0.25 mm with a 0.15-µm film thickness (J&W Scientific, Folsom, CA). Ultrapure helium was the carrier gas at 1.5 ml/min and the GC thermal program was as follows: 60°C for 1 min, 15°C/min to 200°C, 5°C/min to 260°C, held for 10 min. The peak areas were recorded by XCalibur-Qual Browser software (Thermo Quest Inc. Schaumburg, IL). FAME peaks were identified by comparison of retention times and mass spectra with authentic FAMEs (NuCheck Prep, Inc.), 13-methylmyristic, and 14-methylhexadecanoic acids (Sigma-Aldrich Inc.). Each FAME was quantified based on the quantity of spiked internal standard (C23:0).

Collection of 2-Hydroxyl Fatty Acids

OH-FAMEs were eluted into a separate fraction by eluting the column with 6 ml of ethyl acetate based on the method of Jeska & Vetter (2008) after normal (nonpolar) FAMEs were eluted by 5% ethyl ether in hexane.

BSTFA Derivatization of 2-OH Fatty Acids

Silylation of hydroxyl fatty acids from the Formosan subterranean termite by BSTFA followed the method of Laine et al. (1974) with a slight modification. Briefly, ethyl ester containing OH-fatty acid methyl esters (OH-FAMEs) was dried down completely under nitrogen gas flow on a warm pan and 100 µl of BSTFA (Sigma-Aldrich) was added to each vial. The reaction for trimethylsilylation was allowed to proceed for 30 min at room temperature. BSTFA was dried down as described above and 200 µl 0.05% BHT in chloroform was added to the vial and stored at –20°C until analyses. FAMEs and fatty acid methyl-d_3 esters were analyzed by GC-MS as described above. FAME peaks were identified by comparison of retention times and mass spectra with authentic FAMEs (NuCheck Prep, Inc.), 13-methylmyristic and 14-methylhexadecanoic acids (Sigma-Aldrich Inc.). Each FAME was quantified based on the quantity of spiked internal standard (C23:0). OH-FAME peaks were

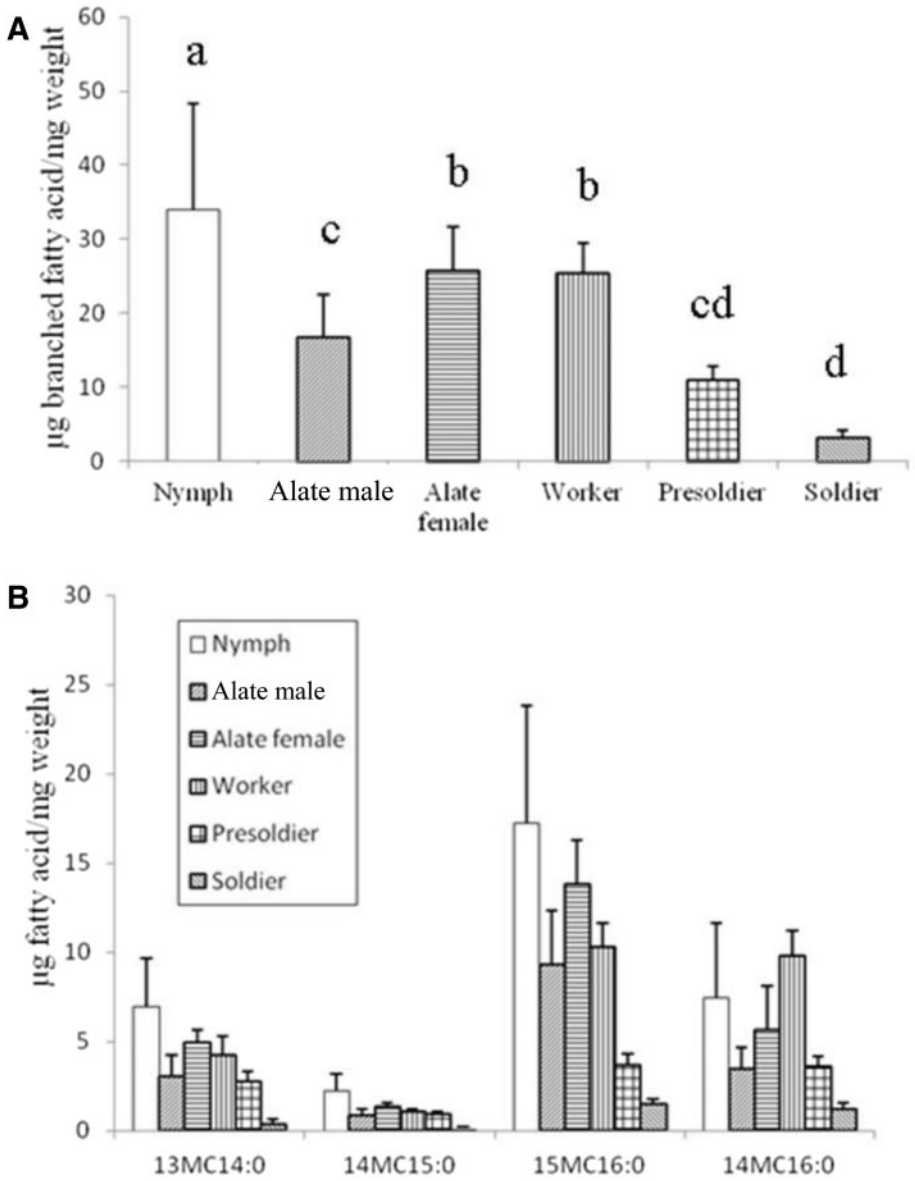

Fig. 4. Branched fatty acids in *C. formosanus*. (**A**) Total branched fatty acid content in nymph, alate, worker, presoldier, and soldier. (**B**) Branched fatty acid profiles of nymph, worker, presoldier, and soldier and alates including males and females. Means with different letters are significantly different ($P < 0.05$). Data are presented as mean ± standard deviation.

identified by comparison of retention times and mass spectra with authentic OH-FAMEs (Matreya LLC.). Each OH-FAME was quantified based on the internal standard (2-OH C23:0).

Analysis of Data

A Proc Mixed Procedure model (SAS 9.1 software, SAS Institute, Cary, NC) was used to analyze the data. A one-way analysis of variance was performed to determine if there were significant differences in fatty acid content among nymphs, workers, presoldiers, soldiers, and alates. All data were judged at $\alpha = 0.05$.

Results

Major Fatty Acids in *C. formosanus*

Quantitatively and qualitatively different fatty acid compositions were found from nymphs, alates, workers, presoldiers, and soldiers. The total fatty acid content in both nymphs and alate females was about 1.5-fold higher than that of alate males, about 2-fold higher than that of workers, and 6-fold higher than that of presoldiers, whereas it was about 12-fold higher than that of soldiers (Fig. 1A) which had the lowest amount of fatty acids. Alate females had a significantly higher amount of total fatty acid compared to that in alate males ($P = 0.005$). Oleic acid (C18:1) was the predominant fatty acid in nymphs, alates, workers, presoldiers, and soldiers. The major difference in total fatty acid in the developmental stages and the castes was due in large part to difference in oleic acid (C18:1), stearic acid (C18:0), linoleic acid (C18:2), and palmitic acid (C16:0) (Fig. 1B and C). Seventeen different fatty acids occurred commonly in all developmental stages and castes, whereas soldiers had a significantly higher amount of docosanoic acid (C22:0) ($P < 0.0001$) and among the castes, two unique long-chain fatty acids, lignoceric acid (C24:0) and hexacosanoic acid (C26:0) (Figs. 1B and 2A and B).

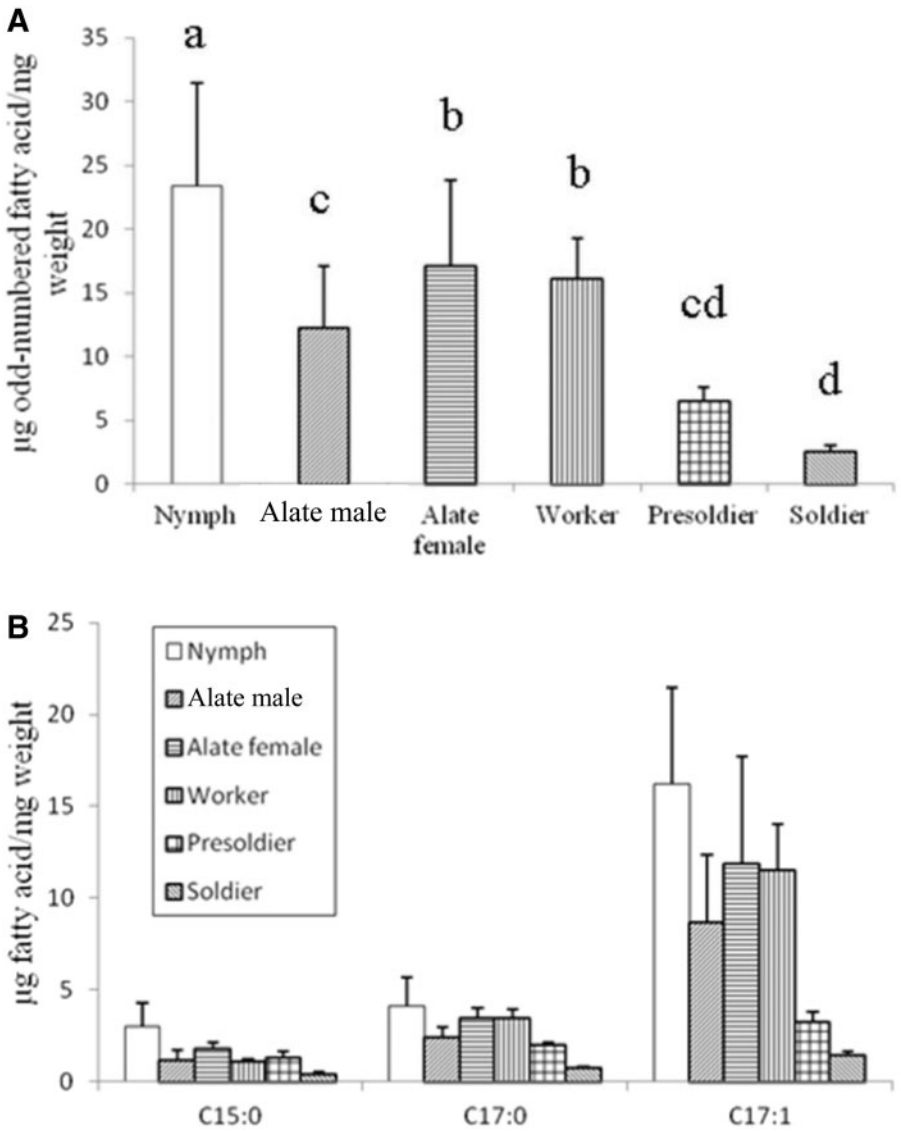

Fig. 5. Odd-numbered fatty acids in *C. formosanus*. (**A**) Total odd-numbered fatty acid content in nymph, alate, worker, presoldier, and soldier. (**B**) Odd-numbered fatty acid profiles of nymph, worker, presoldier, and soldier and alates including males and females. Means with different letters are significantly different ($P < 0.05$). Data are presented as mean ± standard deviation.

Branched Fatty Acids in *C. formosanus*

Four branched fatty acids were found in all termite samples. Peak I, retention time at 11.65 min, and peak V at 13.80 min (Fig. 3B) were identified as 13-methyl myristic acid and 14-methyl palmitic acid, respectively, after comparison with the retention time and ion spectra of standards (Fig. 3A, C, and D). The major fragment ions for 13-methyl C14:0 and 13-methyl-d_3 C14:0 were m/z = 213.2 and 216.2 (shown as 'a' in Fig. 3C and D) [M-43(C_3H_7)], respectively. Fragmentation patterns were as follows: [M-43(C_3H_7)-14(CH_2)$_n$, n = 1,2,··· ···9 and 1–9 corresponding to b–j in the Fig. 3C and D]. Based on the major fragment ions and fragmentation patterns of 13-methyl C14:0 and 13-methyl-d_3 C14:0, peak IV at 13.70 min and peak V at 13.80 min (Fig. 3B) were identified as 14-methyl pentadecanoic acid and 15-methyl palmitic acid. The fragmentation patterns of these compounds were identical to those observed in standard 13-methyl C14:0 and 13-methyl-d_3 C14:0 (Fig. 3E–H). Total branched fatty acid content differed significantly among groups (F, $P < 0.0001$) with nymphs exhibiting the highest total abundance and soldiers the lowest (Fig. 4A). All branched chain fatty acids except 14-methyl palmitic acid were significantly higher in nymphs than in other termite groups (Fig. 4B). Presoldiers also had higher 14-methyl palmitic acid than other castes (Fig. 4B)

Odd Numbered Fatty Acids in *C. formosanus*

Three different normal odd-numbered fatty acids were identified from all forms of the termite after comparison of retention time and ion spectra (data not shown) with the standards. Peak II at 11.95 min, Peak VI at 13.89 min, and peak VII at 14.16 min were identified as C15:0, C17:1, and C17:0, respectively (Fig. 3A and B). Total odd-numbered fatty acid content differed significantly among termite groups ($P < 0.0001$), with nymphs having the highest abundance and soldiers the lowest (Fig. 5A).

All odd-numbered fatty acids in nymphs were significantly higher compared to those in other groups (Fig. 5B, $P < 0.001$).

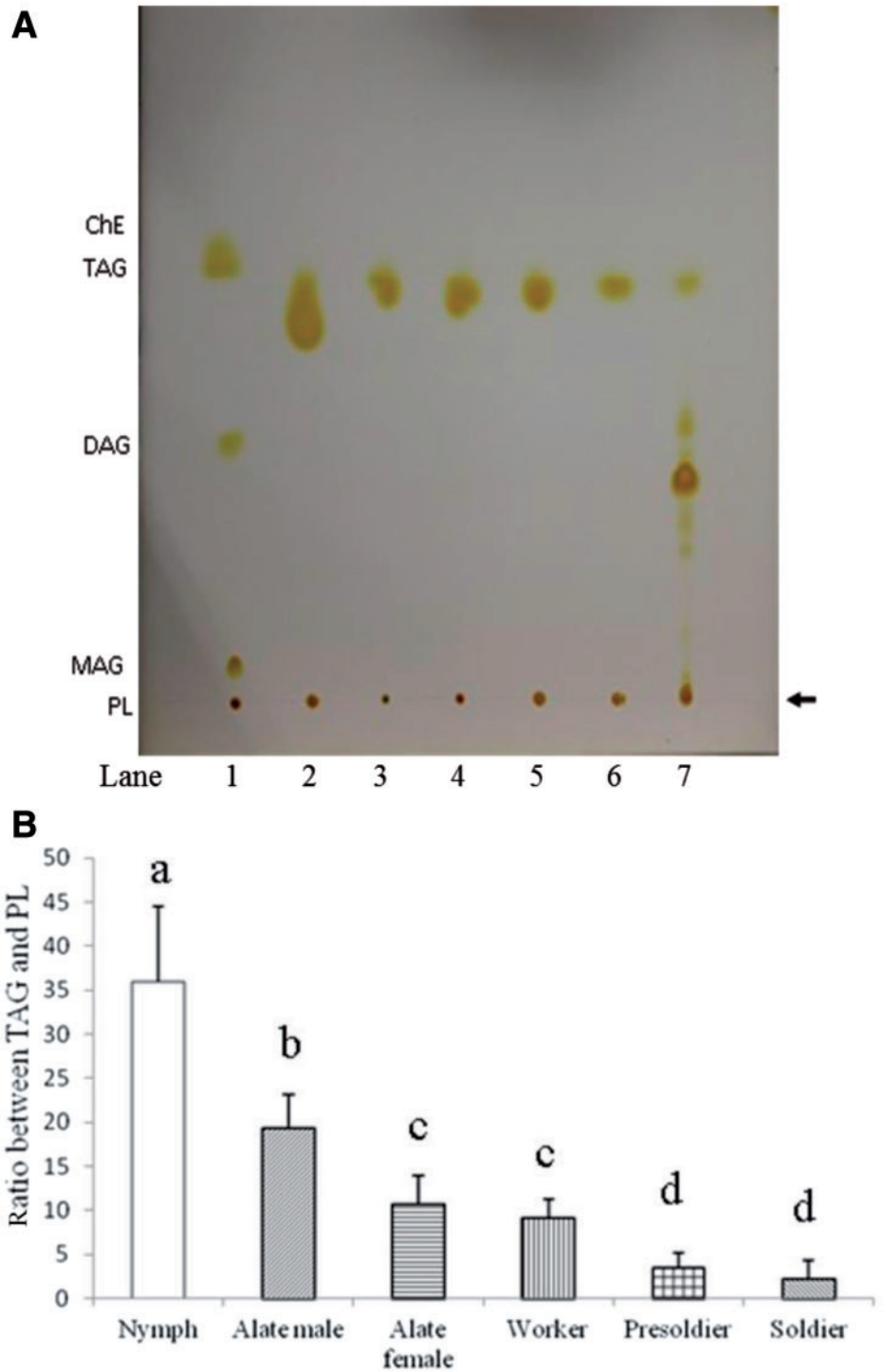

Fig. 6. Analysis of lipids from the Formosan subterranean termite by thin layer chromatography (TLC). (**A**) Separation of lipids from the termites by TLC—Lane 1: Standard mixture; 2: Nymph; 3: Alate male; 4: Alate female; 5: Worker; 6: Presoldier; 7: Soldier. ChE, cholesterol esters; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; and PL, phospholipids. Arrow at lower right side of the figure indicates the origin. (**B**) Ratio between triacylglycerols and phospholipids. Means with different letters are significantly different ($P < 0.05$). Data are presented as mean ± standard deviation.

Storage Lipids From *C. formosanus*

The ratio of TAGs/PLs in nymphs was significantly higher than that in the other developmental stages and castes of the termite and ratios in presoldiers and soldiers lower than others (Fig. 6B; $P < 0.0001$). The ratio in alate males was significantly higher than that in alate females and workers ($P < 0.001$) even though alate males had significantly lower total fatty acid content than did alate females, and similar total fatty acid content to workers (Fig. 1A). The majority of the fatty acids (~58–90%) both in TAGs and PLs in all developmental stages and castes were composed of even-numbered fatty acids (Table 1). Oleic acid was the major component in both TAGs and PLs. Odd-numbered and branched fatty acids were distributed at different percentages in both TAGs and PLs of all developmental stages and castes of the termite. Soldiers had the

Table 1. Percentage (%) of fatty acid in the lipid classes of the Formosan subterranean termite

Fatty acid	Nymph		Alate male		Alate female		Worker		Presoldier		Soldier	
	TAG	PL	TAG	PL	TAG	PL	TAG	PL	TAG	PL	TAG	PL
C14:0	0.78	0.23	0.44	0.47	0.59	0.53	0.57	0.53	0.68	0.67	1.09	0.58
C16:0	15.10	14.55	16.51	14.19	14.89	12.82	16.06	15.25	16.77	10.96	18.87	14.62
C16:1	2.13	1.82	1.61	1.43	2.12	2.22	2.17	2.30	2.38	2.23	2.09	3.28
C18:0	11.66	13.41	11.59	11.04	11.11	11.46	13.41	10.63	12.34	11.32	12.41	17.57
C18:1	49.20	47.79	52.01	62.95	51.64	49.73	44.06	33.47	36.42	56.25	22.10	32.41
C18:2	7.46	8.33	2.34	ND	2.37	12.41	4.93	8.83	2.28	0.50	0.76	11.81
C20:0	0.33	0.21	0.13	0.09	0.27	0.32	0.35	0.02	0.03	0.01	0.18	1.59
C22:0	ND	0.17	ND	0.11	0.13	0.33	ND	0.38	ND	0.32	0.15	0.65
TPENFA	86.66	86.52	84.63	90.27	83.13	89.82	81.55	71.41	70.90	82.27	57.64	82.52
C15:0	0.86	1.97	0.67	1.34	0.55	1.56	0.57	2.00	1.09	1.67	0.80	2.65
C17:0	2.03	1.68	3.66	1.39	6.20	1.57	6.76	3.05	11.30	2.49	28.41	3.10
C17:1	2.57	3.86	2.74	2.42	2.84	2.51	0.99	10.61	4.35	5.65	3.97	5.88
TPONFA	5.46	7.50	7.06	5.15	9.59	5.64	8.32	15.66	16.74	9.80	33.18	11.62
13MC14:0	1.85	0.50	1.62	0.46	1.12	0.58	1.18	0.67	3.44	0.81	1.19	0.73
14MC15:0	0.60	0.51	0.42	0.56	0.48	0.33	0.46	2.72	0.83	0.90	0.35	0.81
14MC16:0	1.85	1.11	2.08	1.14	1.87	1.06	5.40	2.45	3.90	2.11	3.91	1.70
15MC16:0	3.59	3.87	4.19	2.57	3.80	2.57	3.09	7.03	4.20	4.11	3.73	3.50
TPBFA	7.88	5.98	8.31	4.72	7.28	4.54	10.13	12.86	12.37	7.93	9.18	6.74

Lipids from the termites were separated and analyzed as described in the Materials and Methods. TAG, triacylglycerols; PL, phospholipids; TPENFA, total percentage of even-numbered fatty acids; TPONFA, total percentage of odd-numbered fatty acids; TPBFA, total percentage of branched fatty acids; ND, not detected ($n = 3$).

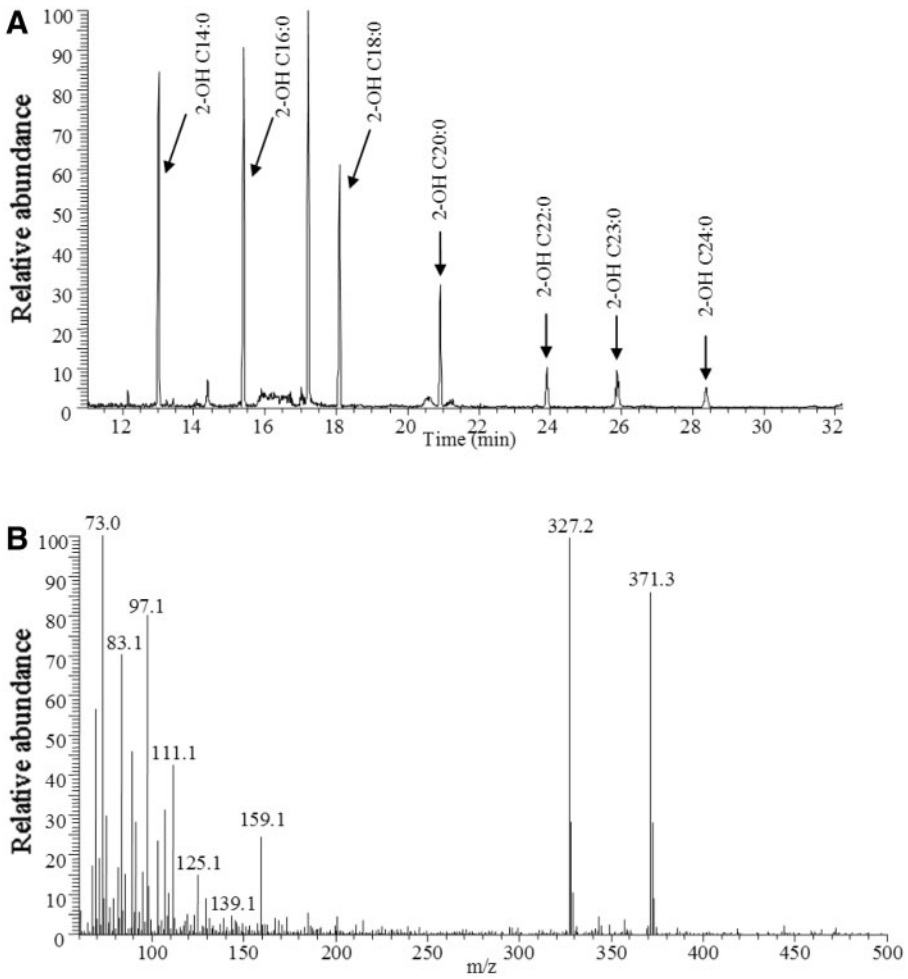

Fig. 7. Analysis of authentic 2-OH fatty acid methyl esters by gas-chromatography and mass spectrometry. (A) Total ion chromatography of the authentic 2-OH FAMEs. (**B**) Ion spectrum of 2-OH stearic acid.

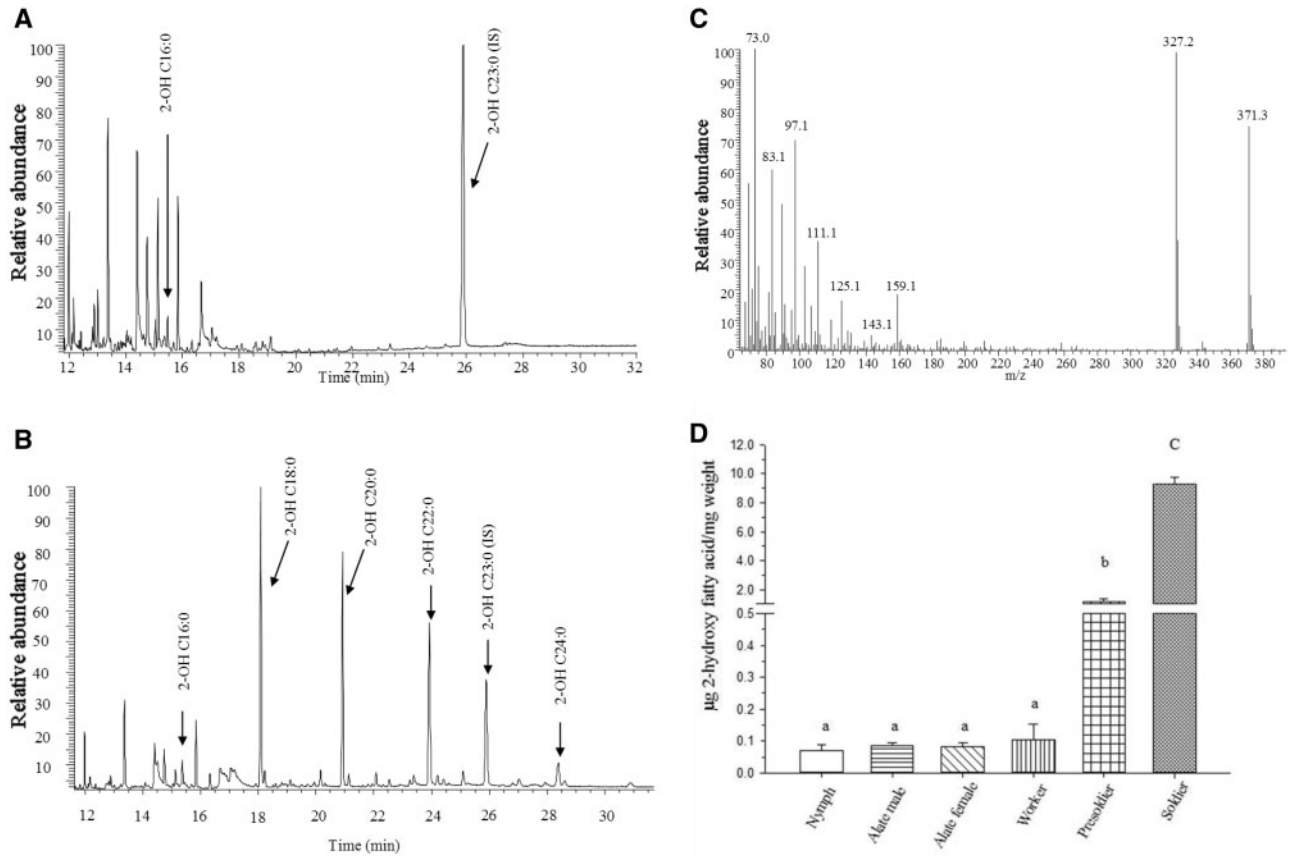

Fig. 8. Analysis of 2-OH fatty acids from the Formosan subterranean termite. 2-OH tricosanoic acid was used as the internal standard (IS) for quantitation. (**A**) Total ion chromatography of 2-OH fatty acid from a worker. A small amount of 2-OH palmitic acid was detected. (**B**) Total ion chromatography of 2-OH fatty acids from a soldier. Five different 2-OH fatty acids were identified from soldiers. (**C**) Ion spectrum of 2-OH fatty acid from a soldier. (**D**) Total 2-OH fatty acids. Means with different letters are significantly different ($P < 0.05$). Data are presented as mean ± standard deviation.

highest percentage of C17:0 (28%) in TAG (Table 1). Lignoceric (C24:0) and hexacosanoic acid (C26:0) were not detected in either TAGs or PLs of soldiers.

Identification and Quantitation of 2-Hydroxy Fatty Acids From *C. formosanus*

Five different 2-hydroxy fatty acids—2-OH C16:0, 2-OH C18:0, 2-OH C20:0, 2-OH C22:0, and 2-OH C24:0—were identified by comparison of retention times and mass spectra with authentic 2-hydroxy fatty acids (Figs. 7A and B, and 8A–C). Two-OH C24:0 was detected only in soldiers (Fig. 8B). All these fatty acids had two high abundance fragment ions—[M-15 $(-CH_3)]^+$ and [M-59 $(-COOCH_3)]^+$—and six commonly occurring ions, m/z = 83, 97, 111, 125, 139, and 159 (Tables 2 and 3).

Total 2-hydroxy fatty acid content differed significantly among castes (Fig. 8D). Amounts were significantly higher in soldiers than in other groups (6.01–7.9-fold vs. presoldiers and 41.7–132.6-fold vs. nymphs, alates, and workers) and the quantity in presoldiers was significantly higher than nymphs, alates, and workers, whereas there was no difference among nymphs, alates, and workers (Fig. 8D). A significant colony effect on amount of 2-hydroxy fatty acids in soldiers was observed but not in workers from different colonies (Data not shown).

Table 2. Major fragmentation ions of 2-hydroxy fatty acids

Fatty acid component	$[M]^+$	$[M-15]^+$	$[M-59]^+$
2-OH-C14:0	330	315	271
2-OH-C16:0	358	343	299
2-OH-C18:0	386	371	327
2-OH-C20:0	414	399	355
2-OH-C22:0	442	427	383
2-OH-C23:0	456	441	397
2-OH-C24:0	470	455	411

Table 3. Common ions of 2-hydroxy fatty acids

Chemical structure	Corresponding ions
CH_3-$(CH_2)_2$-CH = CH-CH_2	83
CH_3-$(CH_2)_3$-CH = CH-CH_2	97
CH_3-$(CH_2)_4$-CH = CH-CH_2	111
CH_3-$(CH_2)_5$-CH = CH-CH_2	125
CH_3-$(CH_2)_6$-CH = CH-CH_2	139
CH_3-$(CH_2)_2$-CH = O^+-Si$(CH_3)_2$-CH_2	159

Discussion

Our results showed that nymphs and alate females had the highest fatty acid content and alate males and workers had the second highest fatty acid content compared to soldiers and presoldiers. However, the ratio between triacylglycerols and phospholipids in nymphs was significantly higher than that in other forms, probably because nymphs store more lipids for transformation to alates. The TAG/PL ratio in

alate males was significantly higher than that in alate females although total fatty acid content in alate females was significantly higher than in alate males. This result suggests that alate males store more triacylglycerols than alate females, for unknown reasons.

In this study, we identified 19 different fatty acids in the Formosan subterranean termite. An earlier study by Mauldin et al. (1972) described 12 different fatty acids from workers of Formosan termites and identified eight. Mauldin et al. (1972) also reported an unidentified C15 fatty acid, which could be either the singly branched C14:0 or C15:0 found in our study. It was interesting that the fatty acid profiles of workers and presoldiers were similar, whereas that of presoldiers and soldiers were significantly different. A developmentally regulated fatty acid synthesis scheme may co-occur with the molt from presoldier to soldier. Our results show that among the castes, soldiers have two unique fatty acids, lignoceric acid (C24:0) and hexacosanoic acid (C26:0), which were previously identified as major free fatty acid components of the frontal gland defense secretion (Chen et al. 1999, Ohta et al. 2007). We did not detect these fatty acids in TAGs and PLs of soldiers (Table 1). These free fatty acids were either biosynthesized in, or otherwise incorporated into the frontal gland of the soldiers.

The role(s) of the fatty acids in the frontal gland secretion of soldiers is not understood (Chen et al. 1999, Mao et al. 2005, and Ohta et al. 2007). Mao et al. (2005) found that there was no behavioral or toxic effect on red imported fire ants when they were treated with some free fatty acids; however, they stimulated Formosan soldier formation from workers. Consistent with others, our results showed that oleic acid (C18:0) was the predominant fatty acid in alates, workers, nymphs, presoldiers, and soldiers. Four branched fatty acids and four odd-numbered fatty acids were found from all developmental stages and castes. To our knowledge, this is the first characterization and quantitation of monomethyl branched and odd-numbered fatty acids in termites. Both branched and odd-numbered fatty acids were distributed at different percentages in TAGs and PLs of the different developmental stages and castes in the termite indicating dual functions in energy storage and membrane components. Nymphs had significantly greater amounts of branched fatty acids compared to alates, workers, presoldiers, and soldiers, indicating that branched fatty acids might be involved in caste development, especially in the nymphal stage. Kniazeva et al. (2004) demonstrated monomethyl branched fatty acids played an essential role in *Caenorhabditis elegans* development. They found that *C. elegans* development was arrested when enzymes for branched fatty acid biosynthesis were suppressed, and recovered when the arrested animals were fed monomethyl branched fatty acids. Although the role of these fatty acids in development of *C. elegans* and other ecydysozoans such as insects is unknown, they may serve as a precursor for long-chain and very long-chain methyl-branched alcohols, which may be precursors for pheromone biosynthesis in the Formosan subterranean termite as has been reported in the German cockroach, *Blattella germanica* (L.) (Chase et al. 1992, Tillman et al. 1999). They described that the German cockroach biosynthesized the sex pheromone, 3,11-dimethylnonacosan-2-one, by elongation of a methyl-branched fatty acyl-CoA moiety followed by decarboxylation, hydroxylation, and oxidation.

Nelson et al. (1989, 1990a,b) described long-chain and very long-chain methyl-branched alcohols in the cabbage looper, *Trichoplusia ni* (Hubner), and the tobacco hornworm, *Manduca sexta* (L.). These alcohols are produced by a fatty acid reductase on long-chain fatty acids. The distribution of odd-numbered fatty acids in organisms is rare; therefore, researchers usually utilize odd-numbered fatty acids such as C15:0 and C17:0 as internal standards for quantitation of fatty acids. Our results show that show that nymphs, alates, workers, presoliders, and soldiers of Formosan termites contain 3–23 μg/mg (~4–6% of total fatty acid) odd-numbered fatty acids including C15:0, C17:0, and C17:1. It suggests that the building blocks for fatty acid biosynthesis in the termite are both acetyl-CoA, from which are produced even-numbered fatty acids, and propionyl-CoA, from which are produced odd-numbered fatty acids. The occurrence of odd numbered fatty acids in termites is very interesting and could arise from symbionts in the gut tract producing propionate, as do the symbionts in cattle, which also have odd numbered fatty acids. The 14-methyl palmitic acid and the 13 methyl myristic acid could be unused byproducts of the precursors for the 3- and 2-methyl hydrocarbons, which account for about 30% of this termite's hydrocarbons (Haverty et al. 1996). This study showed that in Formosan termites, the TGA/PL ratio was high in alates, probably due to their higher energy requirements, that soldiers have a high abundance of 2-hydroxy fatty acids compared to those in other developmental stages and castes, and previously unreported odd numbered and branched fatty acids were differentially found in the termite castes. More work is needed to determine their occurrence outside of the Formosan termites, and to determine what roles all of these compounds may play in behavior and development.

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