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Cuticular chemistry of two social forms in a facultatively polygyne ant (Hymenoptera: Formicidae: *Formica truncorum*)

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In monogyne (single queen) ant colonies, worker aggression against intruders, including newly-mated sister queens (gynes) seeking readoption, prevents the development of multi-queen colonies. Some ant species, however, produce multiple queen colonies (polygyny) via the adoption of new gynes in response to ecological, genetic and social parameters. Cuticular chemistry mediates nestmate recognition as well as plays a role in intra-colonial regulation of reproduction. Although aggression against intruders is diminished in some polygyne species, apparently due to depauperate allelic diversity at loci that code for recognition cues or a loss of receptor sites, the mechanism underlying the development of polygyny is largely unknown. Here, we examine the cuticular chemical profiles of new gynes at several stages post-emergence, males and workers from monogyne and polygyne colonies in the facultatively polygyne ant *Formica truncorum*. Individuals from the two colony types were chemically distinguishable, as were gynes according to developmental stage post-emergence (callow *versus* mature) and with respect to wing presence and mating status. Overall profile complexity did not differ between populations. These results suggest an association of cuticular hydrocarbon profiles with dispersal tendencies and that the disparate tendency of workers from monogyne and polygyne colonies to adopt new gynes is due to a complex interaction between gyne cuticular chemistry and differential worker sensitivity to those cues.

Introduction

Cooperative behavior is widespread among diverse animal groups (Hepper 1991), and builds on the ability to recognize and discriminate between kin and non-kin (Hamilton 1963) or between cooperators and cheaters (Dawkins 1976). Membership as well as position within cooperative societies is conveyed in vertebrate and invertebrate taxa by an array of complex visual, auditory, seismic or olfactory signals. Some social insects display an extreme form of cooperation, eusociality, whereby some individuals largely forgo personal reproduction and

labor on behalf of breeders (Hamilton 1963). Social cohesion is maintained through an elaborate system of recognition and communication that involves surface and glandular chemical cues to regulate societal functioning. Whereas glandular products can alarm, recruit or guide others, surface chemicals, particularly the non-volatile hydrocarbons, are considered important for recognizing nestmates (Obin 1986, Bonavita *et al*. 1987, Espelie *et al*. 1994, Lahav *et al*. 1999) as well as for advertising and recognizing social status (Monnin *et al*. 1998, Monnin & Peeters 1999, Peeters *et al*. 1999). The cuticular chemical profile encompasses both genetic and environmental products (Crozier & Pamilo 1996, Beye *et al*. 1998, Downs & Ratnieks 1999, Silverman & Liang 2001, Giraud *et al*. 2002, El-Showk *et al*. 2010). However, the nestmate badge for many ant species is considered a 'gestalt' odor, a blend of odors to which colony members contribute via trophallaxis and allogrooming (Crozier & Dix 1979, Dahbi & Lenoir 1998a, but *see* van Zweden *et al*. 2009). Any changes in colony constitution (genetics) or environment are therefore reflected in colony profile to which members may easily adjust (e.g., Carlin & Hölldobler 1983, Reeve 1989).

Ultimately, the function of complex recognition systems in eusocial groups seems to be to maintain a balance between the number of breeders and the number of workers as well as the genetic integrity of the colony by providing members with the capacity to reject hetero-colonial intruders (Bourke & Franks 1995, Crozier & Pamilo 1996). Nonetheless, although colonies of many ant species are monogyne (have a single queen) and preserve high levels of relatedness among nest-mates by aggressing intruders, a large number of species vary significantly in colony queen numbers. Multi-queen colonies arise either through the cooperative founding of a new colony by multiple queens (primary polygyny) or when colonies adopt newly mated queens (secondary polygyny). Typically, primary polygyny progresses towards strict monogyny as all but one egg layer are eventually killed after the first brood matures (Hölldobler & Wilson 1977, but *see* Johnson 2004). Secondary polygyny, on the other hand, may lead to colonies that support several to hundreds of reproducing queens and worker cohorts that are only distantly related or unrelated altogether (Bourke & Franks 1995, Keller 1995, Crozier & Pamilo 1996). Both types of polygyny are considered selected responses to ecological hardships (e.g. Rosset & Chapuisat 2007) and involve modifications in the recognition system, such that normally repellent individuals cooperate. With few exceptions (e.g., fire ant [Ross & Keller 1998]), relatively little is known about the mechanisms that underlie this systematic reorganization in social structure or its evolutionary pathway, although it is clear that changes in dispersal motivation and a colony's tendency to adopt inseminated gynes (young queens) are involved. Gyne physical condition prior to dispersal is highly correlated with inferred colony founding potential (Keller & Passera 1989a) and dispersal tendencies (Sundström 1995a, DeHeer *et al*. 1999). New gynes from already polygyne colonies typically show considerable variation in these characteristics (Keller & Passera 1989a, Stille 1996).

Colony queen number varies among and within species. Within a species, polygyny may vary among colonies within populations or entire populations may adopt one or the other strategy (Hölldobler & Wilson 1977, Herbers 1993, Bourke & Franks 1995, Sundström 1995a, 1995b, Chapuisat *et al*. 2004). Reproductive success of breeders in many non-social species depends on the proportion of offspring that emigrate to breed elsewhere. In ants, philopatric individuals have breeding opportunities at the parental site, albeit diminished as compared with successful independent colony founders (Bourke & Franks 1995). The pressing question, therefore, is whether alternative reproductive strategies within a species reflect divergent evolutionary pathways. Furthermore, what mechanisms contribute to these opposing trajectories?

The wood ant *Formica truncorum* is facultatively polygyne, with populations comprised primarily of single-queen colonies, multiple-queen colonies (Rosengren *et al*. 1985, Sundström 1993, 1995a, 1995b) or both colony types (Seppä *et al*. 1995, Sundström *et al*. 2005). Colonies in these well-studied archipelagic populations in southern Finland have been steadily monogyne or polygyne for decades (Rosengren *et al*. 1985, Sundström 1993, 1994, L. Sund-

ström unpubl. data) and differ overall in behavior (Sundström 1995a, 1995b, 1997), physiology (Sundström 1995a, Johnson *et al*. 2005) and sex ratios (Sundström 1995b). Genetic data infer extensive dispersal from monogyne colonies but little or no dispersal from the polygyne colonies (Sundström 1989, 1993). Gene flow between neighboring monogyne and polygyne populations, although minimal, is mediated almost exclusively via males (Gyllenstrand *et al*. 2005). Differential worker sensitivity to foreign (nonnestmate) gynes indicates a potential mechanism by which colony queen numbers are regulated in the two colony types (Sundström 1997).

Examination of ant species that are facultatively polgyne allows us to infer the foundation of divergent reproductive strategies by identifying basic differences in queen behavior, chemistry and physiology. The development of polygyny is yet poorly understood but is likely to involve modification of queen cuticular characteristics in conjunction with other mechanisms. Queens maintain a unique relationship with workers that appears to be mediated by cuticular or pheromonal chemical cues (Jouvenaz *et al*. 1974, Keller 1988, Keller & Passera 1989b, Aron 1992, Chen & Vinson 1999). In multi-queen colonies, cuticular chemical signals associated with ovarian activity determine queen position in reproductive hierarchies (Monnin *et al*. 1998, Liebig *et al*. 2000, Cuvillier-Hot *et al*. 2001, de Biseau *et al*. 2004). Cuticular chemistry also mediates interactions between social-parasite and host queens and workers (e.g., Zimmerli & Topoff 1994, Johnson *et al*. 2001, D'Ettorre *et al*. 2002, Johnson *et al*. 2002). Clearly, the potential exists for workers to discriminate queens on the basis of mating, age and reproductive status (e.g., Hannonen *et al*. 2002). *Formica truncorum*, as a facultatively polygyne species, affords a unique opportunity to uncover potential differences between colony types that may be strong determinants of reproductive strategy (dispersal *versus* natal philopatry). Previous studies have identified specific compounds of the *F. truncorum* worker profile (Nielsen *et al*. 1999, Boomsma *et al*. 2003, Akino 2006, Martin *et al*. 2008a). Here we examine the effect of maturity, mating and dispersal potential, as measured by wing presence at maturity, on the cuticular profile of new gynes from colonies with different social structures to uncover differences that may be associates of reproductive strategy (dispersal *versus* natal philopatry) and may account for differential abilities to establish ties with existing nests. We hypothesize that the degree of chemical profile diversity (i.e., number of compounds) is associated with colony social structure as a proxy for overall dispersal strategy, and that cuticular chemistry reflects gyne maturity, wing presence and mating status. We also examined the chemical profiles of *F. truncorum* males and workers to evaluate general overall colony differences independent of gyne condition.

Material and methods

General procedures

Samples of *Formica truncorum* were collected from three islands in the Hanko region and four islands in the Inkoo region circa 100 km apart in the Gulf of Finland during July 2001 and 2002 (Table 1 and Fig. 1). All islands represent typical archipelago islets on the southern coast of Finland, and support forested patches dominated by *Vaccinium* sp., which serve as winter refugia for the ants from the lichen and moss covered rocky outcrops where summer nests are established (Rosengren *et al*. 1985, Elias *et al*. 2005).

Adult workers and worker and sexual pupae were brought back to Tvärminne Zoological Station and housed in plastic containers (35 × 20 × 20 cm), the sides of which were coated with Fluon® or silicon to prevent ant escapes. Container floors were lined with peat and original nest material to aid moisture retention, and a ceramic tile (10 × 10 cm) was placed in the container center to provide shelter. Adults began to emerge from the cocoons almost immediately after colonies were brought into the laboratory. Males were removed from containers soon after eclosion and placed into separate glass containers to prevent intranidal mating. Laboratory colonies were provided with the Bhatkar and Whitcomb (1970) diet and tuna fish daily, and moisture when necessary. Males had access to nectar from local wild flowers.

To determine whether surface chemistry of gynes differed throughout adult maturation and

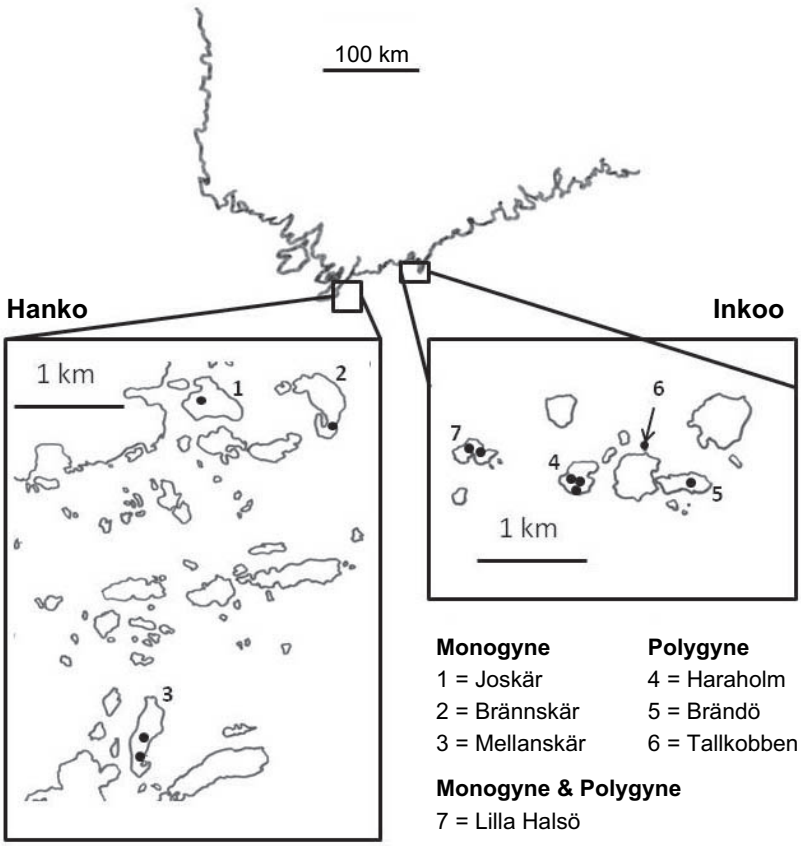

Fig. 1. Locations of the study populations in southern Finland.

with respect to mating and wing condition, we sampled the cuticular chemicals of individual gynes from both monogyne and polygyne colonies upon reaching one of the following stages:

1. Alate callow: newly emerged (within 0–6 hrs) winged virgin gynes;
2. Alate and dealate virgin: mature (7–10 days post emergence) winged and wingless virgin

Table 1. Number of individuals sampled for cuticular chemistry, the islands from which they were collected, and the number of nests on those islands that were sampled.

	Number of nests	Callow	Virgin		Mated		Males	Workers
			Alate	Dealate	Alate	Dealate		
Monogyne								
Brännskär	1	3	4		4	2		
Joskär	1	3	2	5				
Mellanskär	2	9	9		9	4		
L. Halsö-M	1		2	10	1	4	6	4
Total	**4**	**15**	**17**	**15**	**14**	**10**	**6**	**4**
Polygyne								
Haraholm	3	6	6	2	5	2		
Tallkobben	1	4	6		6	1		
Brändö	1	7	3		3			
L. Halsö-P	1		3	8	1	9	6	5
Total	**5**	**17**	**18**	**10**	**15**	**12**	**6**	**5**

gynes;
3. Alate and dealate mated: mature (7–10 days post emergence) winged and wingless gynes that had mated.

To obtain gynes for category 3, we provided copulation opportunities by placing 10–20 females from a single monogyne or a single polygyne colony with 40 males from a single like colony in a circular plastic container (20.5 cm diameter, 13.5 cm height) lined with filter paper and observed them for 45 min between 06:00 and 09:00. One white light and one red light were suspended over the mating chambers to provide additional ambient heat, and the filter paper was moistened slightly with water. When coupling took place, the time to mating was noted and the pair in copula was carefully removed with forceps and placed into a glass jar (7.5 cm diameter, 10 cm height) also lined with filter paper. Here, the pair was left until the female shed her wings or, if wings were not shed, for 30 hours, at which point the cuticular chemicals sampled. The reproductive system of all mature gynes was then dissected and the spermatheca was examined for seminal fluid to verify mating status of queens. Numbers of gynes in each category, males and workers sampled from both colony types are given in Table 1.

Chemical analysis

Workers, gynes, and males were placed in individual 2-ml vials, and a quantity of pentane (99%, HPLC Grade, Rathburn Chemicals [Walkerburn, Scotland]) sufficient to cover the entire body (approximately 0.3–0.5 ml) was added to extract cuticular chemicals. After 10 min, the solvent extract was transferred from the sampling vial with a Pasteur pipette to a new vial and the solvent was allowed to evaporate.

The evaporated extracts were resuspended with 30 μl of *n*-heptane (Riedel-de Haën Laborchemikalien, GmbH and Co., KG, Seelze) and vortexed for 10 sec. Two microliters of each sample were analyzed by gas chromatography/mass spectrometry (GC/MS). The gas chromatograph (HP5890 Series II, Agilent Technologies [Palo Alto, CA, USA] was equipped with an on-column injector and SPME capillary column (Rtx5 ms; 0.5 μl film, 30 × 0.25 mm i.d. [Restek, Bellefonte, PA, USA]). The injector and detector temperatures were 290 °C and 300 °C, respectively. The oven temperature was programmed from 70 °C to 180 °C at 30 °C/min and then from 180 °C to 310 °C at 5 °C/min and held at 310 °C for 20.33 min. Helium was the carrier gas and analyses were performed in splitless mode. Electron impact-mass spectra (EI-MS) were obtained with the GC connected to an HP 5971A MS instrument (Agilent Technologies, Palo Alto, CA, USA) operated at 70 eV. The MS was controlled and the data were analyzed using MSD Chemstation A.03.02 (Scientific Instrument Services, Inc., Ringoes, NJ). Hydrocarbon standards (linear alkenes and alkanes C_{19}–C_{33} [Keele University]) were injected at regular intervals during sample analysis to adjust for any differences in retention times between the two GC/MS instruments used.

Statistical procedures

The relative proportions of cuticular hydrocarbons were computed by dividing the area given for each cuticular hydrocarbon peak by the total integrated peak area of the profile. The peaks of each profile were then autoscaled to ensure equal weight in the analysis (Otto 1999). We used Principal Component Analysis (PCA) to reduce the number of variables considered in the Discriminant Function Analysis models (Joliffe 1986, McGarigal *et al*. 2000). All principal components with an eigenvalue greater than one were used in the discriminant function analysis if the number of principal components was one or more variables less than the number of individuals in the smallest group (Kaiser 1960). Otherwise we used one number of principal components less than the smallest group. We examined the effect of colony type (polygyne/monogyne) and, for gynes, mating (virgin/mated) and wing (alate/dealate) condition, on chemical profile composition using an ANOVA (general linear model) with colony type nested within status. We also compared the number of peaks in the chemical profile and the log-transformed total quantity (log[amt]) of cuticular chemicals, which was adjusted for body

weight (wt) and number of compounds present in the profile (peak). The total chemical quantities were divided by an individual's fresh weight or, if not available, the average fresh weight for that colony and caste, which followed a log-normal distribution, and by the number of compounds. Multiple comparisons were made using Tukey's Honestly Significant Difference tests. Analyses were performed using JMP (SAS Institute, Cary, North Carolina, U.S.A.).

Results

Classification with respect to natal colony type (monogyne/polygyne)

The composition of cuticular chemical profiles comprised a mixture of predominantly *n*-alkanes, methylalkanes and trace amounts of alkenes. The first two principal components revealed overall differences in cuticular hydrocarbon profiles between the two colony types (monogyne *vs*. polygyne) across all castes and gyne age classes (Fig. 2). All males and workers, 84% of callow gynes (n = 32) and 87% of mature gynes (n = 112) were classified correctly with respect to their natal colony type (Table 2). There was no effect of maturity (callow *vs*. mature: Likelihood Ratio χ^2: 0.60, df = 1, p = 0.44), colony type (monogyne vs polygyne: Likelihood Ratio χ^2: 0.34, df = 1, p = 0.56), or an interaction between these (maturity × origin: Likelihood Ratio χ^2: 0.09, df = 1, p = 0.77) on the classification of gynes into the natal colony type. From monogyne colonies, 20% of callow gynes and 16% of mature gynes were misclassified; from polygyne colonies, 18% of callow gynes and 11% of mature gynes were misclassified (Table 2).

Classification with respect to natal colony

Callows were less likely than mature gynes to be classified to the wrong natal colony (callow *vs*. mature: Likelihood Ratio χ^2: 6.2, df = 1, p = 0.01). Only 7% of monogyne callows and 12% of polygyne callows were classified incorrectly as opposed to 36% and 23% of mature gynes,

Table 2. Results of principal components analysis on chemical peaks in profiles of *F. truncorum* gynes, males and workers and of forward stepwise discriminant analysis on the resultant factor scores.

	Number of peaks	Principal Components (eigenvalue < 1)	Percentage of total variance	Number of factor scores in DA	Wilks' λ	F	df	p	Percentage of correctly classified
Differences between colony types									
Callow gynes	37	6	87.91	5	0.69	4.13	3, 28	0.0153	81.25
Mature gynes	45	13	78.33	6	0.39	28.22	6, 105	0.00001	86.69
Males	49	8	96.58	4	0.12	12.29	4, 7	0.003	100
Workers	28	6	94.59	3	0.21	6.36	3, 5	0.037	100
Colony membership by colony types									
Callow gynes									
Monogyne	28	5	87.68	5	0.046	2.7	15, 19	0.02	88.24
Polygyne	27	5	90.4	5	0.67	2.79	15, 25	0.011	93.33
Mature gynes									
Monogyne	43	11	80.05	5	0.17	4.05	27, 129	0.00001	74.05
Polygyne	41	10	80.57	5	0.03	10.87	25, 172	0.00001	82.14

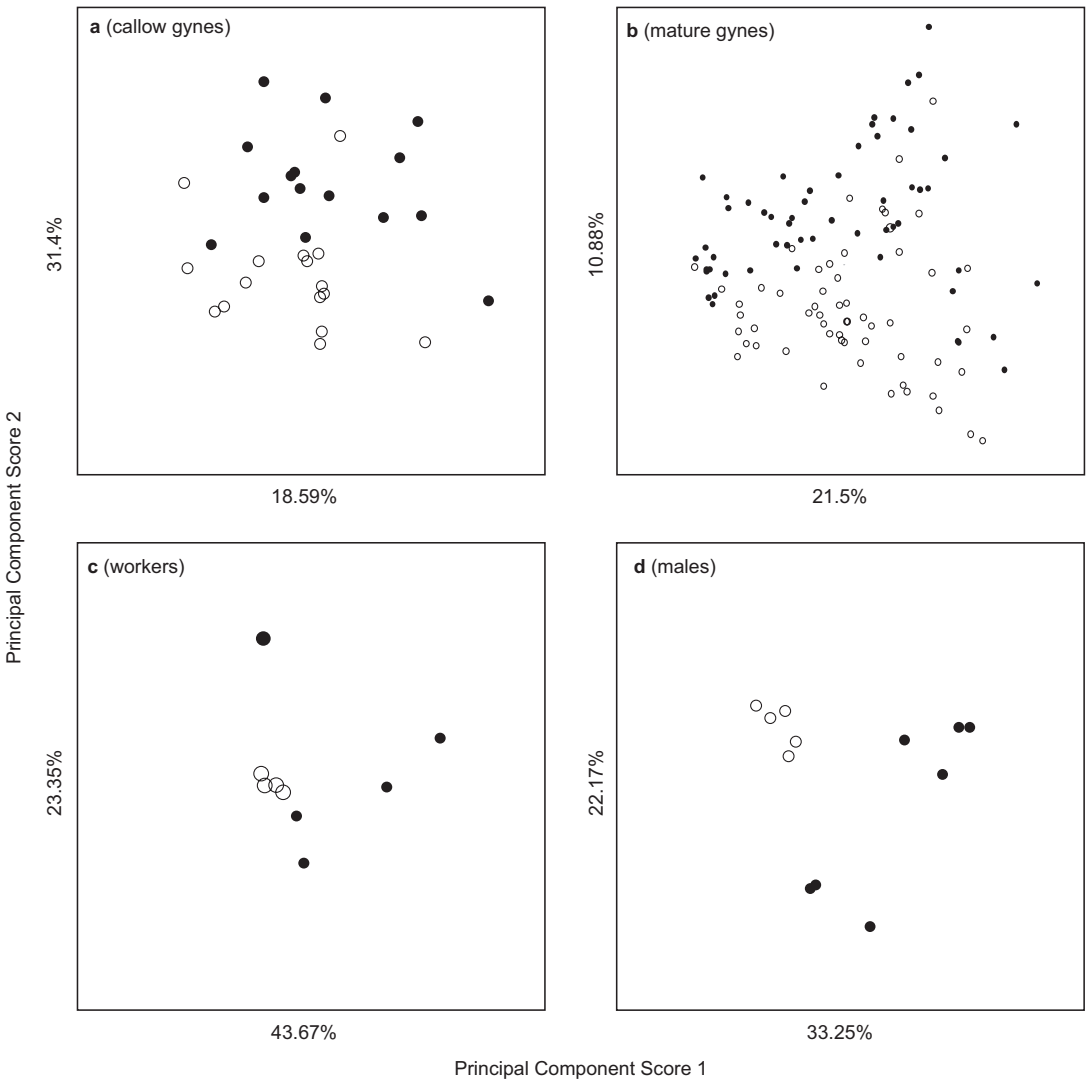

Fig. 2. Plots of the first two principal components reveal inter-colony type differences in chemical profiles of (**a**) callow and (**b**) mature *F. truncorum* gynes, and *F. truncorum* (**c**) workers and (**d**) males from monogyne (●) and polygyne colonies (○).

respectively. Neither colony type (Likelihood Ratio χ^2: 0.0001, df = 1, $p = 0.99$) nor the interaction between maturity and colony type (Likelihood Ratio χ^2: 0.88, df = 1, $p = 0.35$) influenced classifications with respect to natal colony. Misclassifications of gynes from polygyne colonies occurred primarily among colonies belonging to one island, Haraholm, which harbors a unicolonial population with absent colony boundaries (Elias *et al*. 2005), and between Haraholm colonies and the colony from a nearby small island, Tallkobben, which had been artificially established by Haraholm gynes (R. Rosengren pers. comm.). From the monogyne colonies, all individuals from one colony on Joskär Island were misclassified. Because only a single male and a single worker from each colony were sampled, a corresponding analysis on these castes was unjustified.

Cuticular chemical profile characteristics

The overall quantity of cuticular profile compounds differed between gynes from monogyne

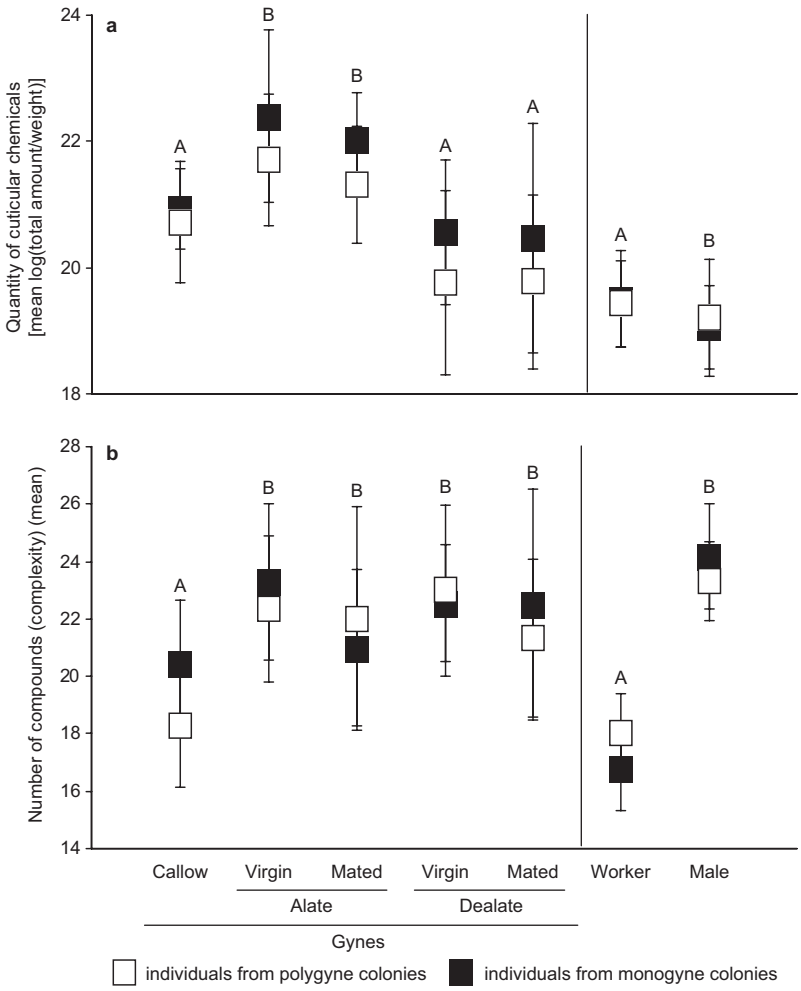

Fig. 3. The average (± SD) (**a**) quantity in and (**b**) complexity of cuticular chemical profiles of *F. truncorum* gynes in different developmental states and of *F. truncorum* males and workers from different colony types.

and polygyne colonies ($F_{1,143}$ = 5.44, p < 0.02) and differed significantly among gyne states ($F_{4,139}$ = 8.22, p < 0.0001). There was no interaction between colony type and gyne state ($F_{4,139}$ = 0.3, p = 0.85) (Figs. 3a and 4). Gynes from monogyne colonies had consistently, slightly higher cuticular chemical amounts ($Mean_{log[amt]/wt/peak\ n}$ = 15.06, SD = 1.35) than gynes from polygyne colonies ($Mean_{log[amt]/wt/peak\ n}$ = 14.66, SD = 1.29), and mature virgin and mated alate gynes had significantly higher amounts than callow or virgin and mated dealate gynes (Tukey's HSD = 2.765) (Fig. 3a). Profile complexity (number of peaks) did not differ according to colony social structure ($F_{1,143}$ = 1.49, p = 0.22) but did differ among gyne states ($F_{4,139}$ = 8.22, p < 0.0001) without respect to colony social structure ($F_{4,139}$ = 1.53, p = 0.12). Callows had significantly fewer peaks than all mature gynes (Tukey's HSD = 2.765) (Fig. 3b).

Workers had significantly higher surface chemical amounts than males ($F_{3,17}$ = 18.33, p = 0.0005). However, there was no difference between colony types ($F_{3,17}$ = 0.25, p = 0.63) nor an interaction between colony type and caste ($F_{3,17}$ = 0.0005, p = 0.98) (Fig. 3a). Male profiles were more complex than worker profiles ($F_{3,17}$= 79.8, p = 0.0001) but there was no difference in profile complexity depending on colony type ($F_{3,17}$ = 0.25, p = 0.63) nor was there an interaction between colony type and caste ($F_{3,17}$ = 3.02, p = 0.1) (Figs. 3b and 5).

Total cuticular chemical quantity increased with number of compounds only in callows from

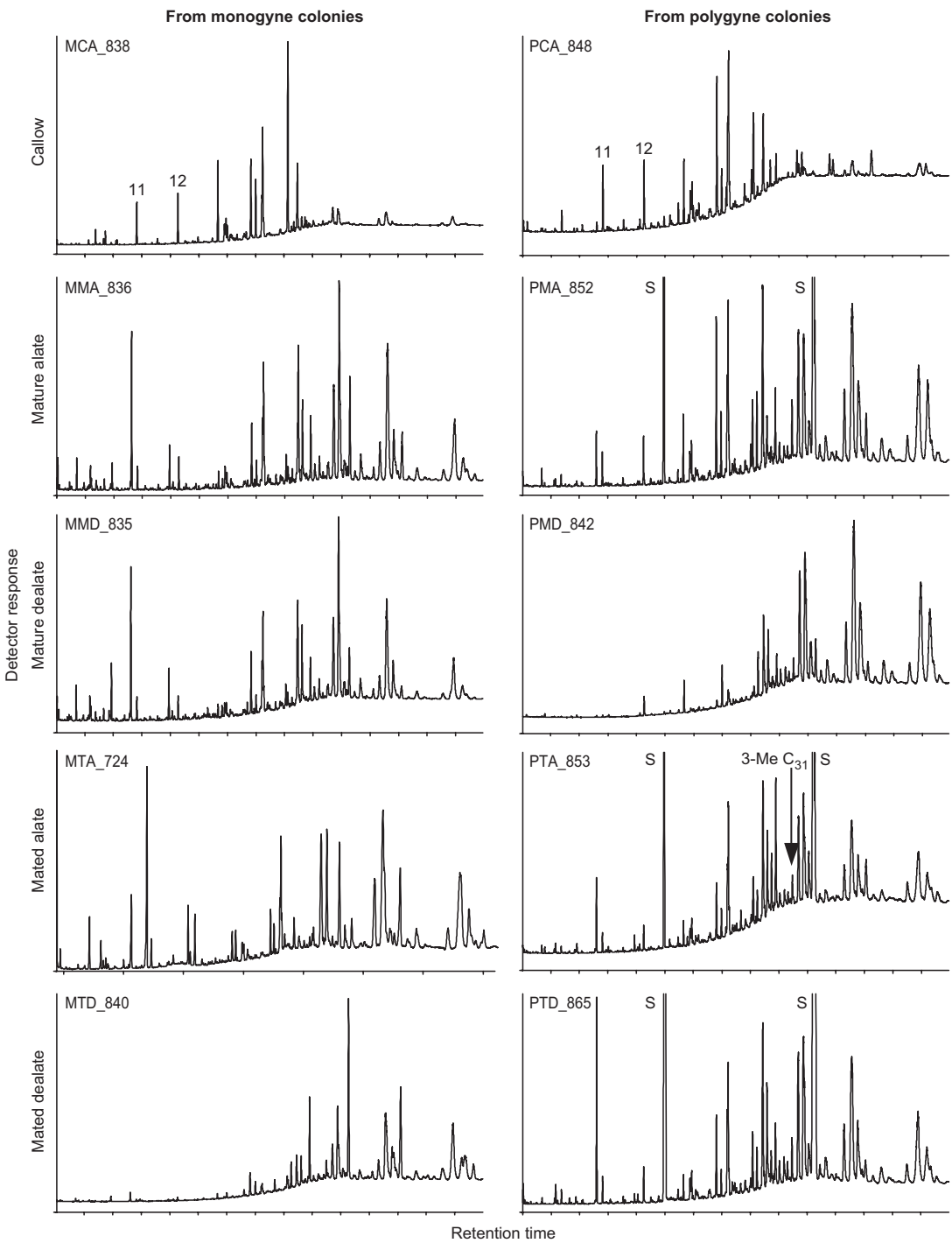

Fig. 4. Representative gas chromatograms of cuticular chemicals found on *F. truncorum* gynes from monogyne and polygyne nests of the various categories sampled for this study. Peak 3-Me C_{31} was found only in gynes from polygyne nests. The change from the callow to mature condition as well as the decrease in profile complexity from the winged to wingless condition is evident. The 'S' identifies peaks of the internal standard.

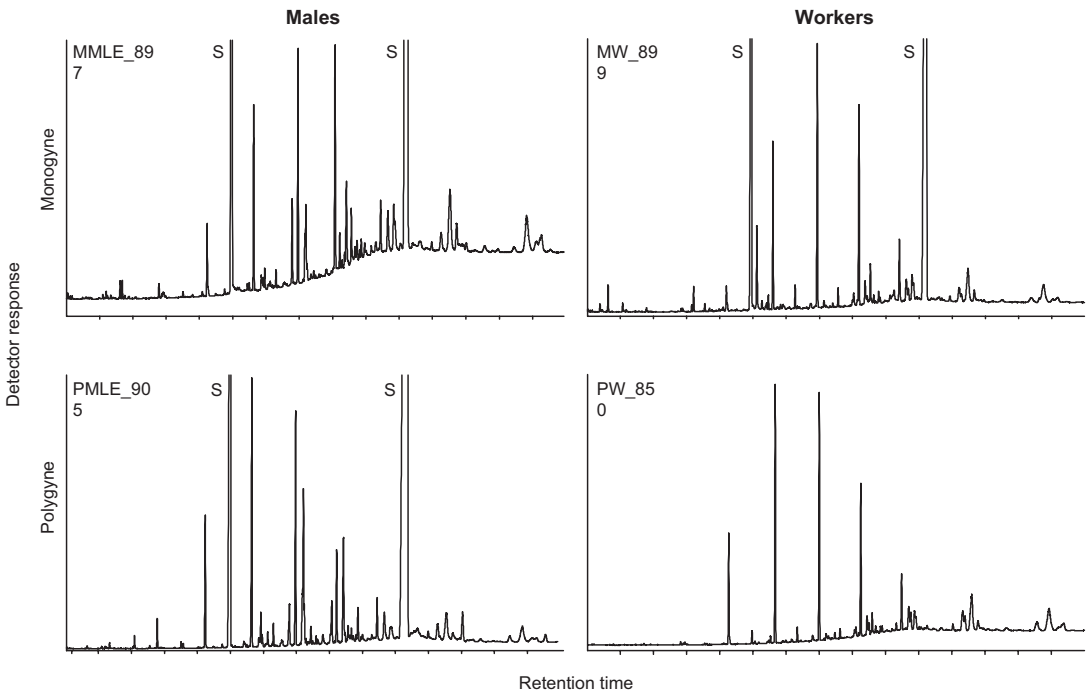

Fig. 5. Representative gas chromatograms of cuticular chemicals found on *F. truncorum* workers and males from monogyne and polygyne nests. Note the relative simplicity of the worker profiles as compared to the more complex profile of the males. The 'S' identifies peaks of the internal standard.

monogyne colonies (linear regression: r^2_{adj} = 0.31, df = 1,13, p = 0.02) and in dealate virgins from monogyne (r^2_{adj} = 0.26, df = 1,13) and polygyne (r^2_{adj} = 0.37, df = 1,8) colonies (p < 0.04) (Appendix 1). Gyne profiles showed notable differences in compounds between developmental stages: Callows lacked appreciable numbers and quantities of most of the relatively low molecular weight peaks in their profiles (< C_{21}) as well as many of the high molecular weight peaks (> C_{32}) (Fig. 4). Two of these low molecular weight peaks (peaks 11 and 12, Fig. 4), found in 70% of mature gynes and in all males, were detected in only five (15%) of the callow gynes and probably indicate more advanced development at sampling time. Peak 3-Me C_{31} was found only in mature polygyne females.

Mature gynes were generally distinguishable with respect to mating status (virgin vs. inseminated) and wing condition (alate vs. dealate) on the basis of overall chemical profiles in both monogyne and polygyne populations (Wilks' λ: monogyne = 0.17, $F_{27,129}$ = 4.05, p < 0.00001; polygyne = 0.15, $F_{27,132}$ = 4.46, p < 0.00001) (Table 3). Neither of the two colony type gynes (monogyne and polygyne) was significantly more often misclassified with respect to mating status or wing status (Fisher's exact test: p = 0.67) (Table 3). In general, misclassifications occurred in all directions with no specific bias (ranging from 20% to 60% misclassified).

Discussion

Cuticular chemistry is one constituent in, or consequence of, the evolution of divergent social structures in ants. Our study is among the first to incorporate colony social structure within an examination of the developmental trajectory of cuticular chemical profiles in new gynes. The wood ant *F. truncorum* in southern Finland forms colonies that embody different life history strategies (to disperse or be philopatric). We found significant differences in the chemical profiles between colonies of the two social types, monogyne and polygyne, as well as among colonies in general. We also found differences among

females at different stages and between sexes. For gynes, there appears to be quantitative as well as qualitative changes in surface chemistry at different stages of development post-emergence. Quantity and complexity of surface chemicals increased from the callow stage, and then quantity decreases after dealation (Figs. 3 and 4). Workers and males had low quantities of surface chemicals (Fig. 3a). However, males had a large number of compounds whereas the worker profiles were relatively simple (Figs. 3b and 5). There were no differences in complexity or quantity between the two populations. The post-eclosion changes in new gynes clearly provide workers with the potential to identify age and mating status of young queens as well as colony membership.

Previous work on *F. truncorum* worker chemistry revealed lower surface hydrocarbon diversity (Nielsen *et al*. 1999) than this and other studies (Akino 2006). In particular, the study by Akino (2006) revealed the ample presence of compounds with chain lengths beyond C_{37}. Our method precluded detection of these long-chained compounds, which arguably could contribute to the palette of recognition cues in this species. Nonetheless, with the subset of all compounds used in this study, we were able to detect changes associated with individual maturation, colony-specific recognition cues, and overall differences between the two colony types. The extent to which long-chained compounds are part of the recognition/discrimination palette in the range we detected is unclear, but we expect the inclusion of these compounds would only serve to amplify the findings of this study.

Typically, the single-queen colony condition can be maintained through worker behavior for extended periods (Rosengren *et al*. 1985), whereas colony genetic integrity is decreased with the advent of additional queens, the effect of which is sometimes ratcheting (Fortelius *et al*. 1993, Rosengren *et al*. 1993). We also expected simpler chemical profiles in gynes from polygyne colonies as information content in generic profiles is potentially limited and lower chemical diversity is considered a possible precondition for the shift away from established colonial boundaries (Tsutsui *et al*. 2003, but *see* Vasquez *et al*. 2008, Schmidt *et al*. 2010). Although the chemical profiles between colonies of the two social types differed, the overall profile complexity, as measured by the number of peaks present on the cuticle, did not. This suggests that the information pool available for discrimination is similar for the two colony types. Although allelic diversity of *F. truncorum* polygyne colonies, which are polydomous or sometimes unicolonial, is lower than in the monogyne colonies (Elias *et al*. 2005), we found no corresponding decline in chemical complexity. Hence, the loss of colony boundaries is not inevitably associated with reduced chemical diversity. The accuracy with which individuals were assigned to

Table 3. Classification matrix of chemical profiles of mature gynes from monogyne and polygyne colonies according to wing and mated status using forward stepwise discriminant function analysis.

	Mated status	Wing status	Percentage of correctly classified	Virgin		Mated	
				Alate	Dealate	Alate	Dealate
Monogyne							
Observed	Virgin	Alate	81.25	13			3
		Dealate	87.5	1	14		1
	Mated	Alate	73.3	3	1	11	
		Dealate	50	1	4		5
	Total		75.44	18	22	11	6
Polygyne							
Observed	Virgin	Alate	77.78	14	3		1
		Dealate	60	3	6		1
	Mated	Alate	73.33	2		11	2
		Dealate	69.23	2		2	9
	Total		71.43	21	9	13	13

their colony type despite similar chemical profile complexity indicates that colony type identification lies in relative quantitative differences of shared compounds and, for mature polygyne females, the qualitative difference in the exclusive presence of 3-Me C_{31}. Alternatively, the absence of differences here suggests that the degree of cuticular chemical profile complexity is not responsible for the divergent responses of workers from colonies with different social structures (Sundström 1997), or that only a limited number of cuticular compounds mediate inter-colonial interactions (Martin *et al*. 2008b).

Undoubtedly, there is a genetic component to chemical profile content (Gotzek & Ross 2007), as there is to size (Bargum *et al*. 2004). However, chemical profiles are dynamic and vary with physiology (de Biseau *et al*. 2004, Johnson & Gibbs 2005), age (Cuvillier-Hot *et al*. 2001), abiotic conditions (Wagner *et al*. 1998) and season and year (Nielsen *et al*. 1999, Boomsma *et al*. 2003). The monogyne and polygyne colonies were located largely in two different areas. Thus, both divergences due to isolation per se and colony-type chemical specificity could account for the observed differences between monogyne and polygyne colonies. Earlier genetic studies on these same study colonies indicate no significant differentiation between colony types (Sundström 1993, Elias *et al*. 2005). This suggests that insufficient time has passed to allow genetic, and possibly also chemical, divergence between the two colony types. If this is so, there appears to be an association between colony type and cuticular chemistry, the source of which may be genetical, environmental, or physiological. Given the manifest chemical differences between the mono- and polygyne colony types despite common rearing conditions strongly suggests that this may indeed be true. Homogenization at neutral loci may be maintained by gene flow through one of the sexes (Gyllenstrand *et al*. 2005) and, therefore, could explain the lack of genetic divergence between the study populations (Sundström 1993, Elias *et al*. 2005). Further investigation designed to directly associate genetic composition with qualitative and quantitative differences in CHCs is clearly necessary to clarify whether this relationship is real.

Ecological and dietary differences may also account for chemical differences between colony types. However, our samples were taken from both colony types at the same time of the same years from islands with similar habitats and reared under similar laboratory conditions. Furthermore, the profiles from the monogyne Lilla Halsö-M colony surrounded by polygyne colonies (Lilla Halsö-P) were consistent with the monogyne chemical form. Instead, there may be inherent differences in colony types due to the differential investment necessary to accommodate existing colony queen number (Silverman & Liang 2001, van Zweden *et al*. 2009, El-Showk *et al*. 2010, van Zweden & d'Ettorre 2010). For example, if queen number differs, queen diet quality may differ between colony types. Indeed, we found significant differences in total body weight, thorax muscle weight, mature alate wing length (unpub. data) and thoracic musculature (Johnson *et al*. 2005) between colony types. This suggests that additional caste- or state-specific selection pressures such as physiological necessities may be as important a determinant of chemical profile complexity as allelic diversity.

Clear colony-specific signals in the *F. truncorum* chemical profiles allowed for reasonably precise assignment of gynes to their natal colony. Hence the reduced discrimination against nonnestmates in *F. truncorum* polygyne colonies (Sundström 1997) is not a function of low cue diversity but may be due to differential behavior. The misclassifications of individuals between the unicolonial colony on Haraholmen Island and the colony on Tallkobben Island that was artificially established with gynes from Haraholmen indicate furthermore that colony chemical integrity can be maintained within lineages for generations.

Quantity and complexity of surface chemicals in gynes increased from the callow stage, and then decreased after dealation in surface chemistry within ten days of emergence (Fig. 3). Similar to several other ant species (e.g., *Linepithema humile* [de Biseau *et al*. 2004], *Gnamptogenys striatula* [Lommelen *et al*. 2006]), mating alone did not impact *F. truncorum* profiles. All individuals in our study with high potential for exposure to abiotic elements (mature gynes and males) had

more complex profiles with numerous methyl-branched alkanes (Fig. 3b). Of the gynes, those with dispersal potential (mature alate gynes) had the highest quantity of cuticular chemicals (Fig. 3a), whereas quantities in dealate gynes were low presumably due to the loss of wings and the associated compounds. Callow gynes had low quantity and complexity of surface chemicals with compounds in a relatively narrow range of low to medium molecular weight compounds whereas the higher molecular weight compounds (> C_{32}) emerged with age (Fig. 4). Johnson and Gibbs (2005) reported similar corresponding changes in virgin alate and mated dealate queens of *Pogonomyrmex barbatus*. Males and workers had low, although different, amounts of surface chemicals and differed significantly in complexity (Fig. 3a and 5). The worker profiles were similar to those found by Martin *et al*. (2008a) and dominated by linear alkanes with only few methyl-branched alkanes in trace amounts. Akino (2006), however, used a more tolerant capillary column that was able to withstand higher temperatures, and found additional high molecular weight compounds in the worker profiles. Presumably, this new methodology will reveal compounds outside the range in which most cuticular hydrocarbons have been found for *F. truncorum* and other ant species. A comparison between the compounds found by Akino (2006) on workers and those we found on sexuals shows that the chemical diversity of sexuals is nevertheless greater than that in workers (Appendix 2).

A shift in compound molecular weight has been associated with young gynes becoming reproductively active (Liebig *et al*. 2000). Other studies have found the reverse is true (Endler *et al*. 2006) or that specific compounds change in quality or quantity but not in necessarily in molecular weight upon becoming reproductive individual (Dahbi & Lenoir 1998b, Hartmann *et al*. 2005, Lommelen *et al*. 2006, Lommelen *et al*. 2010). Our study demonstrates that changes can take place with maturation prior to ovarian development and suggests that these may be functional changes that increase successful colony-founding by increasing water repellency and decreasing water loss in dispersers (Lockey 1980, 1988).

Conclusions

This study is the first to incorporate colony social structure within an examination of the developmental trajectory of cuticular chemical profiles in young gynes prior to the onset of oviposition. Our results show differences in cuticular chemistry of new gynes, males and workers from different colony types and that the developmental trajectory of individual gynes is reflected in their cuticular profile independent of their fecundity. Profile complexity did not differ between the two colony types, which suggests that workers assess the mating status and provenance (own or foreign) of gynes by quantitative differences in conjunction with wing presence or absence. Thus, the divergent reaction of workers from monogyne and polygyne *F. truncorum* colonies is likely due to differential behavior rather than the loss of informative cues on the gyne cuticle.

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Appendix 1. Results of linear regression of number of cuticular chemical peaks on amount of cuticular chemicals in *F. truncorum* gynes of different status, males and workers from monogyne and polygyne colonies. *p* values indicating significances are set in boldface.

Category	Colony type	r^2_{adj}	*F*	df	*p*
Callows	Monogyne	0.343	8.31	1, 13	**0.013**
	Polygyne	0.069	2.18	1, 15	0.16
Virgin alates	Monogyne	–0.08	0.42	1, 15	0.53
	Polygyne	–0.036	0.4	1, 16	0.534
Virgin dealates	Monogyne	0.34	8.19	1, 13	**0.013**
	Polygyne	0.33	5.45	1, 8	**0.048**
Mated alates	Monogyne	0.09	2.36	1, 13	0.15
	Polygyne	–0.074	0.029	1, 13	0.868
Mated dealates	Monogyne	0.05	1.47	1, 8	0.26
	Polygyne	–0.034	0.6	1, 11	0.453
Males	Mongyne	0.043	1.23	1, 4	0.33
	Polygyne	0.06	1.33	1, 4	0.31
Workers	Mongyne	–0.31	0.064	1, 3	0.82
	Polygyne	–0.49	0.01	1, 2	0.92

Appendix 2. Table of compounds found on the cuticle of *F. truncorum* gynes, males and workers in our study and of workers in Akino's (2006) study.

Callows	Mature gynes	Males	Workers	Workers (Akino 2006)
	$C_{19:1}$			–
n-C_{21}	*n*-C_{21}	*n*-C_{21}		–
	$C_{23:1}$			–
n-C_{23}	*n*-C_{23}	*n*-C_{23}		–
n-C_{25}	*n*-C_{25}	*n*-C_{25}	*n*-C_{25}	*n*-C_{25}
				$C_{25:1}$
	11-MeC_{25}	11-MeC_{25}		9-, 11-, 13-MeC_{25}
	9-MeC_{25}	9-MeC_{25}		3-MeC_{25}
	7-MeC_{25}	7-MeC_{25}		–
				n-C_{26}
		$C_{27:1}$		$C_{27:1}$*
n-C_{27}	*n*-C_{27}	*n*-C_{27}	*n*-C_{27}	*n*-C_{27}
11-MeC_{27}	11-MeC_{27}	11-MeC_{27}		9-, 11-, 13-MeC_{27}
9-MeC_{27}	9-MeC_{27}	9-MeC_{27}		3-MeC_{27}
7-MeC_{27}	7-MeC_{27}	7-MeC_{27}		–
11,x-, 15,x-diMeC_{27}	11,x-, 15,x-diMeC_{27}	11,x-, 15,x-diMeC_{27}		–
9-, x-, 14-, x-C_{27}	9-, x-, 14-, x-C_{27}	9-, x-, 14-, x-C_{27}		–
				n-C_{28}
				x-MeC_{28}
unknown		unknown		
$C_{29:1}$	$C_{29:1}$	$C_{29:1}$	$C_{29:1}$	$C_{29:1}$
n-C_{29}	*n*-C_{29}	*n*-C_{29}	*n*-C_{29}	*n*-C_{29}
11,13-diMeC_{29}	11,13-diMeC_{29}	11,13-diMeC_{29}		9,x- and/or 11,x-diMeC_{29}
9-MeC_{29}	9-MeC_{29}	9-MeC_{29}		x-MeC_{29}
7-MeC_{29}	7-MeC_{29}	7-MeC_{29}		–
unknown				
				n-C_{30}
	10,12-, 16,x-diMeC_{30}	10,12-, 16,x-diMeC_{30}		–
				x-MeC_{30}
				$C_{31:1}$*
	n-C_{31}		*n*-C_{31}	*n*-C_{31}
9-MeC_{31}				
7-MeC_{31}				
	13,15-diMeC_{31}			11,x- and/or 13,x-diMeC_{31}
	13,17-, 15,x-diMeC_{31}			
11-, x-MeC_{31}				11-, 13-MeC_{31}*
3-MeC_{31}	3-MeC_{31}		x-MeC_{31}	3-MeC_{31}
	10-MeC_{32}			–
	$C_{33:1}$			$C_{33:1}$
				$C_{33:2}$
	n-C_{33}	*n*-C_{33}	*n*-C_{33}	–
	11-MeC_{33}	11-MeC_{33}	11-MeC_{33}	11-, 13-, 15-MeC_{33}
	9-MeC_{33}	9-MeC_{33}	9-MeC_{33}	
	7-MeC_{33}	7-MeC_{33}	7-MeC_{33}	
				11,x-, 13,x- and/or 15,x-diMeC_{33}*
				–
	$C_{35:1}$	$C_{35:1}$	$C_{35:1}$	$C_{35:1}$
	11-MeC_{35}	x-MeC_{35}	x-MeC_{35}	11-, 13-, 15-, and 17-MeC_{35}
	9-MeC_{35}	x-MeC_{35}	x-MeC_{35}	
	7-MeC_{35}	x-MeC_{35}		
				x,x,x-triMeC_{35}
				11,x-, 13,x-, 15,x- and/or 17,x-diMeC_{35}*
				x, x-diMeC_{36}
	C_{37}	C_{37}		–
	11-MeC_{37}	x-MeC_{37}	x-MeC_{37}	11-, 13-MeC_{37}
	9-MeC_{37}	x-MeC_{37}	x-MeC_{37}	
	7-MeC_{37}	x-MeC_{37}		
				11,x-, 13,x-, 15,x- and/or 17,x-diMeC_{37}*
				11,19,25-triMeC_{37}*

*Found in appreciable amounts by Akino (2006).