frame magnetic fields may also prove advantageous (*27*). The window is thus opened to other potential applications where xenon may be adsorbed in materials and on surfaces, or dissolved in blood and other biological systems.

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21. Cross-relaxation between the two spin systems, **I** and **S**, is described by the Solomon equations (*28, 29*):

$$\frac{d}{dt} I_z = -\rho_I(I_z - I_0) - \sigma_{IS}(S_z - S_0)$$
$$\frac{d}{dt} S_z = -\rho_S(S_z - S_0) - \sigma_{SI}(I_z - I_0) \quad (2)$$

where I_z and S_z are the z components of the **I** and **S** spins and I_0 and S_0 are the equilibrium values; ρ_I and ρ_S are the autorelaxation rates. The cross-relaxation rates, σ_{IS} and σ_{SI}, are related to each other by

$$\sigma_{IS} = \frac{I(I+1)N_S}{S(S+1)N_I}\sigma_{SI} \quad (3)$$

where N_I and N_S are the concentrations of **I** and **S** spins in the liquid. At time t_0, I_z goes through either a minimum or a maximum, depending on the sign of $[S_z(t_0) - S_0]$. At this time, $\frac{d}{dt} I_z = 0$, and from Eq. 2 one obtains

$$\frac{I_z(t_0) - I_0}{I_0} = -\frac{\sigma_{IS}}{\rho_I}\frac{\gamma_S S(S+1)}{\gamma_I I(I+1)}\frac{[S_z(t_0) - S_0]}{S_0} \quad (4)$$

where the high temperature approximation for S_0 and I_0 was used, $I_0 \approx I(I+1)\hbar\gamma_I B_0/(3k_B T)$, $S_0 \approx S(S+1)\hbar\gamma_S B_0/(3k_B T)$, where $\hbar$ is the Planck constant, k_B the Boltzmann constant, B_0 the external magnetic field strength, and T the temperature of the liquid. σ_{IS} can be estimated by means of Eq. 3 and the relation between σ_{SI} and ρ_S^{SI}, the part of the autorelaxation rate of spins **S** due to their dipolar interaction with spins **I**. In the fast motion limit,

$$\sigma_{SI} = \frac{S(S+1)}{2I(I+1)}\rho_S^{SI} \quad (5)$$

From Eqs. 3 and 5 one then obtains

$$\sigma_{IS} = \frac{N_S}{2N_I}\rho_S^{SI} \quad (6)$$

22. In order to estimate the cross-relaxation rate σ_{IS} (Eq. 6), we use $\rho_S^{SI} = (390\ \mathrm{s})^{-1}$, determined from the value of the spin-lattice relaxation times (T_1) of ^{129}Xe in benzene and fully deuterated benzene solution (*20*). The ratio of ^{129}Xe (80% enriched) to ^1H in C_6H_6 is $N_S/N_I = 0.0015$, using the solubility data of Xe in benzene (0.011 mole fraction at 1 atm and 25°C) (*30*). One obtains $\sigma_{IS} = 1.9 \times 10^{-6}\ \mathrm{s}^{-1}$, independent of the concentration of **I** spins (*29*) and assumed to be the same in the partially deuterated benzene.
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34. Supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Sciences Division, of the U.S. Department of Energy under contract DE-AC03-76SF00098. Y.-Q.S. was a Miller Research Fellow, partially supported by the Miller Institute for Basic Research in Science; S.A. was partially supported by the Deutsche Forschungsgemeinschaft; and T. R. was partially supported by the National Science Foundation through grant FD93-11913 administered by the Department of Physics.

7 November 1995; accepted 24 January 1996

Caste-Selective Pheromone Biosynthesis in Honeybees

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Queen and worker honeybees (*Apis mellifera* L.) produce a caste-related blend of functionalized 8- and 10-carbon fatty acids in their mandibular glands. The biological functions of these compounds match the queen's reproductive and the worker's nonreproductive roles in the colony. Studies with deuterated substrates revealed that the biosynthesis of these acids begins with stearic acid, which is hydroxylated at the 17th or 18th position. The 18-carbon hydroxy acid chains are shortened, and the resulting 10-carbon hydroxy acids are oxidized in a caste-selective manner, thereby determining many of the functional differences between queens and workers.

One of the most remarkable aspects of social insect biology is the separation of female colony members into reproductive and nonreproductive castes: queens and workers (*1*). A major difference between the female honeybee castes is the blend of 8- and 10-carbon functionalized fatty acids found in the mandibular glands. Queens have predominantly 9-hydroxy-(*E*)2-decenoic acid (9-HDA) and other acids functionalized at the penultimate (ω – 1) position, among them 9-keto-(*E*)2-decenoic acid (ODA) (*2*). Workers have acids functionalized at the last (ω) position such as 10-hydroxy-(*E*)2-decenoic acid (10-HDA), its saturated counterpart, and the corresponding diacids (*3*). The queen's acids, 9-HDA and ODA, are components of the queen mandibular pheromone (QMP), a powerful attractant of workers and one cue responsible for the retinue of workers around the queen. The queen asserts her reproductive dominance by mediating some worker activities associated with colony growth and reproduction through her QMP signal (*2*, *4*, *5*). The worker-produced acids are secreted in brood food (*6*) where they may function as preservatives (*7*) and larval nutrients (*8*). Thus, queens and workers produce compounds that fit their respective reproductive and nonreproductive roles and differ only in the position of the functional group. Here, we report the elucidation of the biochemical pathway that determines whether a honeybee female will produce

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queen or worker substances in her mandibular glands.

The functionalization pattern of the mandibular acids in queens and workers was thought to be mutually exclusive until ODA was found in the glands of some queenless workers (9) and traces of 9-HDA in queenright workers (*10*). Conversely, mated queens contain small amounts of 10-HDA (*11*). Thus, both castes have the capability of producing 10-carbon ω- and (ω – 1)–functionalized acids, but differ in the selectivity of their biosynthesis. In this study we investigated the biosynthesis of ω- and (ω – 1)–functionalized mandibular acids in both castes to understand how the caste-selective pattern arises.

We propose that caste-determined biosynthesis of mandibular acids can be viewed as a bifurcated three-step pathway (Fig. 1). The ω and ω – 1 branches are established at the first step: hydroxylation of stearic acid at the 18th (ω) or 17th (ω – 1) position. The resulting 18-carbon hydroxy acid chains are shortened to the major components of the blend, 10-HDA and 9-HDA. Oxidation of the ω- and (ω – 1)–hydroxy group to give diacids and keto acids, respectively, completes the process.

To study the biosynthesis of mandibular acids, we applied specifically deuterated test compounds (*12*) to excised mandibular glands and followed the conversion of the acids by gas chromatography–mass spectrometry (GC-MS) (*13*). A comparison of the incorporation of fatty acids of different chain length revealed that stearic acid was incorporated into the hydroxy acids more efficiently than palmitic or decanoic acid (*14*). Further experiments confirmed that stearic acid is the entry point to the biosynthetic pathway of the mandibular acids. Assays with labeled ω– and (ω – 1)–hydroxy acids revealed that the (*E*)2-unsaturated hydroxy acids are derived from their saturated counterparts, and that diacids and keto acids are derived from the corresponding hydroxy acids. No isomerization between 10-HDA and 9-HDA was detected, so the pools of ω– and (ω – 1)–hydroxy acids appear to be independent (*15*).

Because the precursor to the mandibular acids is longer than the products, the biosynthetic pathway must include at least two processes that could occur in either order: the introduction of the hydroxy group (functionalization) and chain shortening. Extracts of mandibular glands contain small but detectable amounts of the 12- and 14-carbon homologs of the mandibular acids. This fact, and the observation that stearic acid is the precursor, prompted us to demonstrate that the glands can shorten labeled 18- and 17-hydroxystearic acid chains to the 12- and 10-carbon length (Fig. 2). No chain elongation occurred when labeled 10-carbon hydroxy acids were applied to the glands, so the longer chain homologs arise through shortening of the 18-carbon hydroxy acids. We used a β-oxidation inhibitor (*16*) [2-fluorostearic acid (*17*)] to confirm that functionalization precedes chain shortening by β-oxidation. Both ω– and (ω – 1)–hydroxy acids with more than 10 carbons accumulated when workers and queens were treated with labeled stearic acid and the inhibitor (Table 1), confirming the order of the two processes.

In the treatments with a β-oxidation inhibitor, labeled 12-hydroxydodecanoic acid was detected, but 12-hydroxy-(*E*)2-dodecenoic acid and the 10- and 8-carbon acids were not (Fig. 3). Further, the 14- and 16-carbon hydroxy acids were present in trace amounts (<5 ng) and showed no significant incorporation of label in the presence or absence of 2-fluorostearic acid. These observations suggest that β-oxidation may be tightly coupled with little release of intermediate acyl coenzyme A (CoA) esters between the 18- and 12-carbon hydroxy acids. A different set of β-oxidation enzymes may continue the chain-shortening process from the 12-carbon length onward. At that point, 2-fluorostearoyl CoA could compete with the intermediate 12-carbon hydroxyacyl CoA esters, thereby inhibiting further β-oxidation.

Formation and chain shortening of ω– and (ω – 1)–hydroxy acids was detected in both castes with labeled stearic acid (Fig. 4). However, both castes bias biosynthesis toward their predominant end product to achieve their characteristic blend. We used data from several experiments to examine each step in the pathway.

We studied functionalization by applying labeled stearic acid and 2-fluorostearic acid to glands and comparing the amounts of labeled 18- to 12-carbon ω– and (ω – 1)–hydroxy acids that accumulated in each caste (Table 1). Because all the hydroxy acids found in the mandibular glands are derived from 17- and 18-hydroxystearic acids, and the latter are the product of the functionalization reaction, the total labeled hydroxy acid accumulated gives an indication of the equivalents

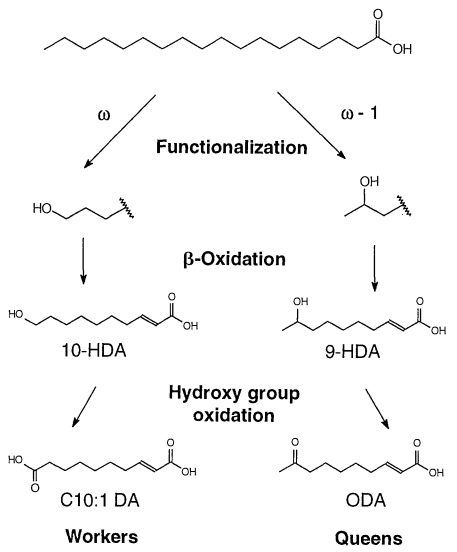

Fig. 1. Biosynthesis of ω- and (ω – 1)–functionalized 10-carbon acids from stearic acid in worker and queen honeybees. C10:1 DA, (*E*)2-decenedioic acid.

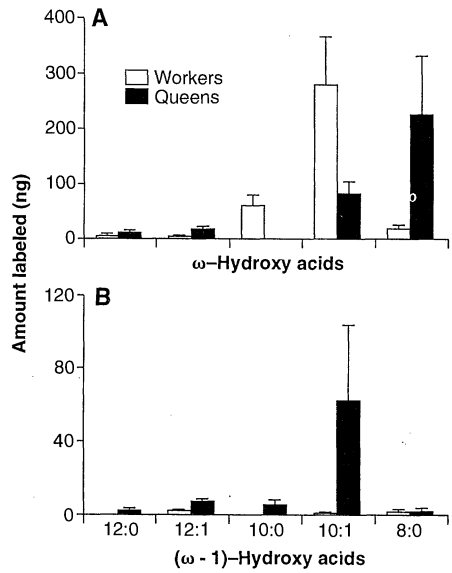

Fig. 2. β-Oxidation of (**A**) 18-D_1 18-hydroxy- and (**B**) 18,18,17-D_3 17-hydroxystearic acids to shorter saturated (:0) and (*E*)2-unsaturated (:1) ω– and (ω – 1)–hydroxy acids, respectively, in workers and queens. The bars represent the mean amount in nanograms of labeled chain-shortened product. Error bars show the standard error (n = 10 for workers and 8 for queens).

Table 1. Hydroxylation is not biased in workers or queens. Amounts of labeled hydroxy acids (HAs), 12 and 18 carbons long, accumulated during 10-min perfusions with D_1 stearic acid as the substrate, in the presence (+) and absence (–) of 2-fluorostearic acid, a β-oxidation inhibitor (Inh.) (n = 8 for both castes). For workers two glands were perfused per replicate, and for queens one gland was perfused per replicate.

Caste	Inh.	ω-HA accumulation (nmol)	(ω – 1)–HA accumulation (nmol)
Workers	+	0.13 ± 0.03*	0.09 ± 0.04
	–	0.06 ± 0.02	0.04 ± 0.01
Queens	+	0.73 ± 0.52*	0.77 ± 0.54*
	–	0.05 ± 0.02	0.06 ± 0.02

*There was a significant difference between the treatments in the presence and absence of 2-fluorostearic acid (pairwise comparison, Kruskal-Wallis $P \leq 0.05$). There was no significant difference between ω– and (ω – 1)–hydroxy acids for any one treatment (Kruskal-Wallis, $P > 0.05$).

of stearic acid hydroxylated during treatment. In both castes, there was no significant difference between the accumulated ω– and (ω – 1)–hydroxy acids, supporting the hypothesis that hydroxylation is not biased.

We studied chain shortening by following the incorporation of label into shorter hydroxy acids from labeled 18- and 17-hydroxystearic acids (Fig. 2). In queens 18-hydroxystearic acid was shortened to the 8-carbon homolog, and 17-hydroxystearic acid to 9-HDA. In workers 18-hydroxystearic acid was shortened to the principal 10-carbon acids, but 17-hydroxystearic acid was shortened only to a small extent. It was not possible to assess whether workers have a low (ω – 1)–hydroxy acid chain-shortening activity or whether they channel 17-hydroxystearic acid into products that were not determined. However, the data revealed that queens and workers have different selectivities of β-oxidation consistent with their respective blends of functionalized acids.

To study the third step, we followed the oxidation of labeled 10-HDA and 9-HDA to the diacid (C10:1 DA) and the keto acid (ODA), respectively, in both castes. Workers and newly emerged virgin queens oxidized 10-HDA to the diacid (*15*), but they did not detectably oxidize 9-HDA to ODA. Studies with worker gland homogenates indicated that hydroxy group oxidation in workers is specific for 10-carbon ω–hydroxy acids (*18*). Our results excluded the possibility that intact worker glands were unable to oxidize 9-HDA because of poor substrate uptake. Mated queens readily oxidized 9-HDA to ODA. Very young queens have small quantities of ODA in their mandibular glands (*19*), so they likely had low 9-HDA oxidizing activity that was not detected.

This study demonstrates that the ω- and (ω – 1)–functionalized acids found in worker and queen mandibular glands are biosynthesized in a branched, three-step pathway (Fig. 1), beginning with the ω or ω – 1 hydroxylation of stearic acid, followed by chain shortening of the 18- and 17-hydroxystearic acids and the oxidation of the ω- and (ω – 1)–hydroxy group. Workers achieve their caste-selective pattern by preferentially chain shortening ω–hydroxy acids to the 10-carbon length and by oxidizing only ω–hydroxy acids to diacids. Queens accumulate more of the 10-carbon (ω – 1)–functionalized acids by preferentially releasing them from β-oxidation at the 10-carbon length and by chain shortening the ω–hydroxy acids to the 8-carbon length. Finally, mated queens readily oxidize 9-HDA to ODA. Thus, caste-specific differences in β- and hydroxy group oxidation steps in the biosynthetic pathway of the mandibular acids lead to the unique chemical signatures of the workers and the queen. These results demonstrate how, in a social insect, caste-determined biosynthesis of isomeric compounds can produce markedly different glandular blends that are responsible for many functional differences between queens and workers.

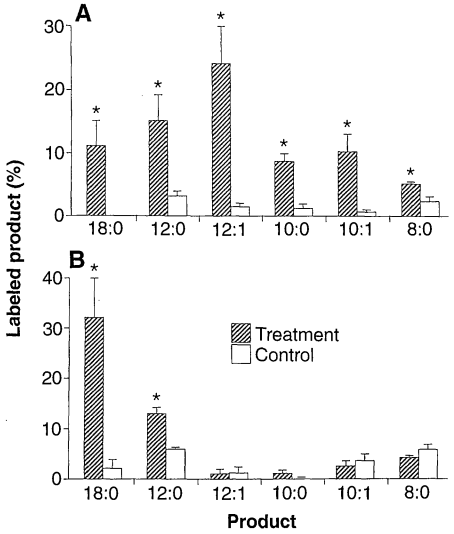

Fig. 3. Incorporation of 12-D_1 stearic acid into ω-hydroxy acids in workers in (**A**) the absence and (**B**) presence of 2-fluorostearic acid. In (A) the controls were glands treated with dimethyl sulfoxide (DMSO), in (B) they were glands treated with 2-fluorostearic acid in DMSO. Values represent the percentage of labeled material as determined by GC-MS; error bars indicate the standard error of the mean (n = 8). Columns marked with an asterisk differ significantly from the corresponding blank ($P < 0.05$, Tukey's test). Similar results were obtained with terminal D_3 stearic acid.

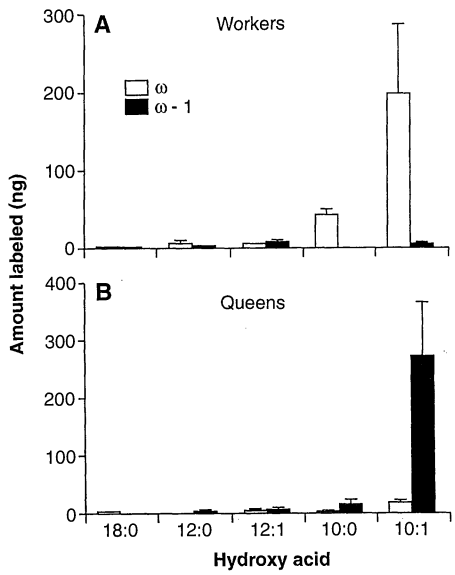

Fig. 4. Incorporation of 12-D_1 stearic acid into ω– and (ω – 1)–hydroxy acids in (**A**) workers and (**B**) queens. Error bars indicate the standard error of the mean (n = 8).

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12. Deuterium was introduced by reduction of carbonyl groups with $NaBD_4$, exchange of protons α to an aldehyde carbonyl with D_2O-pyridine, deprotonation of a terminal alkyne followed by quenching with D_2O, partial or complete reduction of alkynes with D_2, and solvomercuration of terminal alkenes followed by demercuration with $NaBD_4$. The extent of deuteration was monitored by GC-MS and ^1H nuclear magnetic resonance (NMR). In some cases, ^2H NMR was used to confirm the deuteration pattern (*15*). Terminal D_3 octadecanoic acid was obtained from MSD Isotopes (division of Merck Frosst, Montreal, Canada).
13. A solution in dimethyl sulfoxide (DMSO) of the deuterated compound to be tested (0.5 μl of a 40 μg/μl solution) was applied to a freshly dissected mandibular gland (from a 1- to 3-day-old bee). The treatment solution applied was sufficient to cover the outer surface of the gland. In treatments with 2-fluorooctadecanoic acid, the concentration of inhibitor was equal to that of substrate (20 μg/μl). The gland was perfused for 10 min, rinsed with buffer, and extracted with methanol. A sample of the methanol extract was derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and analyzed by GC and GC-MS. The identity of the compounds in the extract was verified by comparison of their retention times and mass spectra with those of synthetic standards. Treatments were compared with glands treated with DMSO. The M-15 ion in the mass spectra of the trimethylsilyl derivatives was used to determine the extent of labeling because it is more prominent than the molecular ion. The percentage of labeled material was calculated from calibration curves prepared with known proportions of labeled and unlabeled standards (*15*).
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20. We thank K. Bray, H. Higo, S. King, P. Laflamme, S. Mitchell, G. Owen, G. Sutherland, M. Tracey, R. Wells, and P. Zhang for technical assistance and T. Pankiw and G. Robinson for reviewing the manuscript. This work was supported by the Natural Sciences and Engineering Research Council of Canada (graduate award to E.P. and Strategic and Operating grants to K.N.S. and M.L.W.) and Simon Fraser University.

30 October 1995; accepted 18 January 1996