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RESEARCH ARTICLE

Honey bee caste lipidomics in relation to life-history stage and the long life of the queen

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ABSTRACT

Honey bees have evolved a system in which fertilised eggs transit through the same developmental stages but can become either workers or queens. This difference is determined by their diet through development. Whereas workers live for weeks (normally 2–6 weeks), queens can live for years. Unfertilised eggs also develop through the same stages but result in a short-lived male caste (drones). Workers and drones are fed pollen throughout their late larval and adult life stages, while queens are fed exclusively on royal jelly and do not eat pollen. Pollen has a high content of polyunsaturated fatty acids (PUFA) while royal jelly has a negligible amount of PUFA. To investigate the role of dietary PUFA lipids and their oxidation in the longevity difference of honey bees, membrane fatty acid composition of the three castes was characterised at six different life-history stages (larva, pupa, emergent and different adult stages) through mass spectrometry. All castes were found to share a similar membrane phospholipid composition during early larval development. However, at pupation, drones and workers increased their level of PUFA, whilst queens increased their level of monounsaturated fatty acids. After emergence, worker bees further increased their level of PUFA by 5-fold across most phospholipid classes. In contrast, the membrane phospholipids of adult queens remained highly monounsaturated throughout their adult life. We postulate that this diet-induced increase in membrane PUFA results in more oxidative damage and is potentially responsible for the much shorter lifespan of worker bees compared with long-lived queens.

KEY WORDS: Social insect, Ageing, Plasmalogens, Membrane peroxidation index, Membrane phospholipids, Polyunsaturated fatty acids, *Apis mellifera*

INTRODUCTION

Social insects represent a promising model for the study of ageing. Ants, termites and honey bees have all evolved a caste system with striking differences in lifespan between genetically identical long-lived queens and short-lived workers (Lucas and Keller, 2014). In honey bees, workers live for weeks whilst queens can live for years. This difference in lifespan readily surpasses, by severalfold, any experimentally achieved lifespan extension (Keller and Jemiely, 2006). In honey bees, a fertilised egg (diploid) can become a worker or a queen dependent upon the size of the honeycomb cell, the level of food fed to the larva and the social context in the hive (Winston, 1987). Unfertilised eggs (haploid) result in the male drone caste. All larvae grow through similar stages immersed in a nutritive fluid (i.e. jelly supplied by the worker caste), but queens emerge after 18 days while workers and drones take 21 and 24 days, respectively, to complete their development from egg to emergent adult (Wang et al., 2015; Winston, 1987).

Bee colonies commonly contain ∼50,000 workers with one queen and a few hundred drones. Division of labour is the hallmark of social insects. Workers perform all tasks related to colony maintenance as they transition through different life-history stages, from in-hive nurses to roving foragers. Nurse bees feed larvae, make and clean honeycomb cells, store incoming pollen and feed newly emergent bees during the first weeks of their adult life. Foragers collect pollen, nectar and water from their environment to provide to the colony (see Winston, 1987, for a full review on life-history stages). Most workers die while on foraging trips at 2–6 weeks of age. In contrast, reproductive queens remain inside the hive for all of their life except for a nuptial flight (during which she mates with several drones) or if swarming. A single queen will mate once and can lay as many as 8 million eggs over her lifetime, a period that can last up to 8 years (AnAge database: http://genomics.senescence.info/species/). Males act as sperm donors for virgin queens and are produced during the warmest months of the year when colonies are likely to swarm and increase their production of queens. The maximum lifespan of drones is estimated at between 20 and 40 days (Page and Peng, 2001) although this short lifespan may involve premature death as drones are reliant upon workers for feeding and are expelled from the hive after the mating season (Rueppell et al., 2005).

Few studies have compared queens and workers to elucidate the extraordinary difference in lifespan, and drones are rarely included in comparative studies. One previous study suggested that differences in nutrition could explain differences in longevity between the female castes (Haddad et al., 2007). This suggestion is based on the type of food consumed after emergence as adult honey bees. Queens are fed a fluid (assumed to be the same jelly they receive as larvae) 'mouth-to-mouth' by worker bees, whereas emergent worker bees begin to consume honey and pollen in the form of 'bee bread' (a mixture of honey, pollen and glandular secretions). Bee bread has a high polyunsaturated fatty acid (PUFA) content (Haddad et al., 2007; Manning and Harvey, 2002; Manning et al., 2007) that increases the PUFA and decreases the monounsaturated fatty acid (MUFA) levels of the membrane phospholipids of worker bees (Haddad et al., 2007). In queens, there is no change in the membrane fatty acid composition, which remains highly monounsaturated throughout life (Haddad et al., 2007; Robinson and Nation, 1970).

PUFAs may influence longevity as they are highly oxidisable (one-thousand times more likely to oxidise than MUFA; Else and

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Kraffe, 2015). The oxidation (peroxidation) of a PUFA can set off an oxidative cascade with the formation of a radical that seeks a further hydrogen atom (with its electron) commonly provided by another bis-allylic bond on the same PUFA molecule, or from surrounding PUFA molecules. This cascade produces lipid hydroperoxides, aldehydes and other by-products that can damage surrounding macromolecules (Halliwell and Gutteridge, 2007). This reaction sequence is often referred to as peroxidation because a peroxyl radical is formed as part of the oxidative process. This autocatalytic process once initiated can be stopped by quenching via antioxidants or by other processes such as self-annihilation, substrate limitation or enzyme activity.

Accumulation of oxidative damage throughout life forms the basis for the oxidative stress theory of ageing (Beckman and Ames, 1998) that was originally proposed as the free radical theory of ageing (Harman, 1956). A variation of this theory, the membrane pacemaker theory of ageing (Hulbert, 2005), emphasises the role of PUFA and membrane lipid peroxidation in free radical damage associated with ageing. Within mammals, birds and some invertebrates, there is a strong relationship between the susceptibility of membranes to peroxidise and maximum lifespan (Hulbert et al., 2017). This correlation exists between highly variable species within animal classes, within similar species or even within variants of the same species with very different longevities (Hulbert et al., 2006) as well as in calorie restriction (Faulks et al., 2006). The common finding in vertebrates and invertebrates of an association between the susceptibility of membranes to peroxidise and maximum lifespan led to the present comprehensive investigation of membrane phospholipids in honey bees. The current study compared the molecular phospholipid composition of three bee castes at six different life-history stages from larva to old adult, from a single free-living colony of bees. We also investigated for the first time the fatty acid composition of male bees and determined what and when changes in molecular phospholipids occur in all castes.

MATERIALS AND METHODS

Source of honey bees and caste sampling

All bees were collected from the same hive at Grenfell, NSW, Australia (GPS 33.901249 S, 148.173194 E). A professional apiarist, Greg Brenner (20 years of experience in the apiary industry), determined the different life-history stages of workers and drones. Criteria used for selection are listed in Table 1. The specific age of workers and drones was not determined. All bees were immediately frozen and stored in dry-ice during transportation to the University of Wollongong, NSW, Australia, where they were subsequently stored at −80°C. Pollen (as 'bee-bread') was collected from the same hive for analysis of fatty acids. Total fatty acid composition of pollen (Table 2) was obtained as previously described (Abbott et al., 2010) using gas chromatography (Shimadzu, Rydalmere, NSW, Australia).

Drones and workers were sampled during the early austral summer (December 2014). Queen bees were manually grafted using eggs laid by the same queen that produced all worker bees used in this study. Briefly, a small larva (first instar) from a worker cell was transferred to a plastic queen cell then moved into a queen-less hive. The plastic queen cells were monitored for 10 days before being transferred into their new hive. Queen larvae were sampled at day 3 (categorised as early larva, n=3) and day 5 (categorised as late larva, n=5). After 8 days, some queen cells were transferred to an incubator (34°C, relative humidity of 70±5%). Pupa queens (day 13, n=5) and emergent queens were sampled after hatching from their respective cells inside the incubator. The remaining sealed queen cells were then transferred into new queen-less hives. As each queen emerged, it was tagged with an acrylic marker (Artline® 400XF) and monitored in natural conditions for the next 12 months. Six queens were randomly sampled for lipid characterisation in December 2015 (categorised as young queen, 12 months old). Previously marked queens of 3 years of age were also sampled in the austral summer of 2014 (n=4). This design reduced any genetic differences between the individuals sampled.

Table 1. Criteria used to determine life-history stage of honey bees (*Apis mellifera*)

Stage	Description
Early larva	Small larva
Late larva	Big larva
Pupa	Cell capped, bee with soft exoskeleton, hairless, eyes developed, wings not developed
Emergent	Collected after emerging from the capped cell, still with soft exoskeleton, no contact with other bees, not yet flown
Young adult workers	Hair fully developed, collected in the hive doing nursing-related tasks, without any pollen on their legs
Old adult workers	Collected near the entrance of the hive, carrying pollen, wings still intact, dark colour
Young adult queens	Marked after emerging and sampled at 12 months
Old adult queens	Marked after emerging and sampled at 3 years
Young adult drones	Hair fully developed, collected in the hive, wings still intact, bright colour, large eyes and larger bodies compared with workers
Old adult drones	Collected inside the hive, wings wearing out, dark colour, large eyes and larger bodies compared with workers

Larvae and pupae of workers were collected from regular-size honeycomb cells. Larvae and pupae of drones were collected from bigger honeycomb cells. Larvae and pupae of queens were collected from plastic cells used to produce queens.

Lipidomics

Analysis of molecular phospholipids was performed as previously described using mass spectrometry (Cortie et al., 2015; Mitchell et al., 2007; Norris et al., 2015). Phospholipid species were extracted from bees (head+thorax+abdomen) as a previous study had shown that membrane fatty acid composition of the three body segments was very similar (Haddad et al., 2007). However, the stinger and attached venom sac of each bee were carefully removed

Table 2. Relative total fatty acid composition of 'bee bread' pollen

Fatty acid	% Total
14:0	4.2±0.4
16:0	21.5±0.9
16:1	0.9±0.7
17:0	6.8±0.6
18:0	2.9±0.3
18:1	5.2±2.1
18:2	28.9±1.2
18:3	28.7±1.0
20:0	Trace
20:1	0.8±0.6
Total SFA	35.5±1.5
Total MUFA	6.9±2.9
Total PUFA	57.7±1.4
Peroxidation index	86.4±2.2

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.
Total lipid fatty acids, n=3. Data are expressed as mol % (means±s.e.m.).

because of the presence of phospholipase A2 in bee venom. Extracts without removal of the stinger and venom sac showed a significant increase in lysophospholipids (data not show).

Bee body mass was measured followed by leg removal to avoid contamination by pollen, which is known to have a high PUFA content. Each bee was homogenised in a Geneworks® homogenisation vial filled with ceramic beads using two passages of 60 s at 6 m s^{-1} in 10 volumes of bulk solvent mix (methanol+internal standard) repeated with methanol only during a second homogenisation to remove all remnants of the bee. For each phospholipid class, an internal standard with acyl chains not detected in honey bees (for details, see Table S1) was added to allow quantification of each phospholipid class. Butylated hydroxytoluene (0.01% w/v) was added to all solvents as an antioxidant to preserve fatty acid composition. To extract lipids, each bee homogenate was vortexed (Mix mate, ThermoFisher, Scoreby, VIC, Australia) for an hour at 4°C in 2 ml of methyl tert-butyl ether (MTBE), then 300 µl of 150 mmol l^{-1} ammonium acetate was added and vortexed for a further 15 min at 4°C (Matyash et al., 2008). Following this, the homogenate was centrifuged at 20,000 ***g*** for 5 min and the lipid-containing MTBE (top) phase was removed and stored under nitrogen gas at −20°C.

Nano-electrospray ionisation mass spectrometry was performed on lipid extracts using a hybrid triple quadrupole linear ion trap mass spectrometer (QTRAP® 5500 AB Sciex, Framingham, MA, USA) equipped with an automated chip-based nano-electrospray source (TriVersa Nanomate™, Advion Inc., Ithaca, NY, USA). On the day of analysis, samples were diluted with methanol:$CHCl_3$ (2:1) containing 5 mmol l^{-1} ammonium acetate to an optimal concentration of approximately 10 µmol l^{-1} of total phospholipids. Samples were loaded into 96-well plates, centrifuged (10 min, 2200 ***g***) and directly infused into the mass spectrometer. Spray parameters were optimised at a gas pressure of 2.8 kPa with a voltage of 1.2 kV and 1.1 kV for positive and negative ion modes, respectively.

Glycerophospholipid MS/MS prediction tool (www.lipidmaps.org) was used to make target lists and converted to targeted ion lists used in Lipidview™ (version 1.3, AB Sciex). Lipidview™ software was set at a mass tolerance of 1 Da, with a minimum intensity of 1% and a minimum signal-to-noise ratio of 10. Positive precursor ion scans were used to quantify phospholipid molecules for lysophosphatidylcholine (LPC), phosphatidylcholine (PC), lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PE) and phosphatidylserine (PS). A negative precursor ion scan was used to quantify phospholipid molecules for phosphatidylinositol (PIn). Negative precursor ion scans were used to identify fatty acids using a custom-made spreadsheet in Microsoft Excel 2014 (Microsoft Corporation, Redmond, WA, USA). A list of all precursor ion scans used is provided in Table S2. Phospholipids were quantified at the sum composition level (e.g. PC 36:2) for each respective phospholipid head group before the molecular phospholipid level was determined from fatty acid scans (e.g. PC36:2 can be PC18:0_18:2 or PC18:1_18:1).

The sn-1 and sn-2 positions of each fatty acid on the phospholipid molecules could not be identified using the current method and isomeric phospholipid species containing alkyl ethers (termed O=) or vinyl ethers (plasmalogens, termed P=) could not be differentiated. Those two isomeric molecules were interpreted as plasmalogen in the current study. Individual phospholipid molecules were quantified by comparison with internal phospholipid standards of the same class after correction for isotope contribution. Phospholipid molecules are reported as nmoles of molecular phospholipid per milligram of bee (nmol mg^{-1}). Phospholipid structure is reported using the nomenclature described by Liebisch et al. (2013).

Fatty acid composition

Membrane fatty acid composition, as a percentage of total fatty acid, was calculated from the fatty acid compositions of the quantified phospholipid molecules present in the extract using a formulated Microsoft Excel spreadsheet. Total fatty acids combined all molecular phospholipids quantified by Lipidview™. Fatty acids are expressed as mol % of total fatty acids.

Peroxidation index

Membrane peroxidation index (PI) of whole-bee lipid extracts was calculated as the sum of bis-allylic methylene groups per 100 fatty acids according to the equation:

$$\mathrm{PI} = (\Sigma\%\ \text{di-PUFA} \times 1) + (\Sigma\%\ \text{tri-PUFA} \times 2) + (\Sigma\%\ \text{tetra-PUFA} \times 3) + (\Sigma\%\ \text{penta-PUFA} \times 4) + (\Sigma\%\ \text{hexa-PUFA} \times 5). \quad (1)$$

Statistical analysis

Membrane phospholipids were compared between castes and at different life-history stages using a two-way analysis of variance. All analyses were performed with R software (version 3.2.2).

RESULTS

Body mass

During development, body mass increased from a few milligrams as larva to 100–296 mg as adults in the different castes (Table 3). Queen larvae were the largest of the castes, being 2–3 times larger than those of workers and drones (Table 3). As pupa, queens and drones were of similar mass, being 2.5- to 3-fold larger than worker bees. Worker bees reach their maximum body mass as young adults (~159 mg) whereas older foragers had a body mass similar to that at emergence (~100 mg). At emergence, drones possessed the largest body mass followed by queens, then workers but body mass reduced with age in drones (~22%), whereas queens increased in size to become the largest of the adult caste, reaching a maximum of 284 mg at 12 months.

Total fatty acids

MUFA were the dominant membrane fatty acid throughout the different life-history stages of all bee castes, accounting for between 65% and 80% of fatty acids (Fig. 1B). Worker bees continually decreased their MUFA levels throughout their development,

Table 3. Body mass of different bee castes

	Workers		Queens		Drones	
Life stage	*n*	M_b (mg)	*n*	M_b (mg)	*n*	M_b (mg)
Early larva	10	1.6±0.2^a	3	16.4±6.1^b	9	21.9±1.7^b
Late larva	10	76.5±4.9^a	5	207.6±35.6^b	10	85.7±12.7^a
Pupa	10	106.3±5.3^a	5	255.2±5.2^b	7	295.7±17.0^b
Emergent	9	105.2±1.0^a	5	183.3±7.3^b	5	269.0±6.2^c
Young adult	10	158.7±5.7^a	6	283.6±6.7^b	8	210.5±6.7^c
Old adult	10	100.0±4.0^a	4	255.2±6.8^b	10	207.4±1.8^c

Young adult queens were 12 months old. Old adult queens were 3 years old. Body mass (M_b) data are means±s.e.m. Lowercase letters indicate a difference between the castes for the same life-history stage (e.g. body mass is similar between early larva queens and early larva drones but significantly higher than that of early larva workers). Level of significance was set at $P<0.05$ when comparing castes (e.g. early larva workers, early larva queens, early larva drones).

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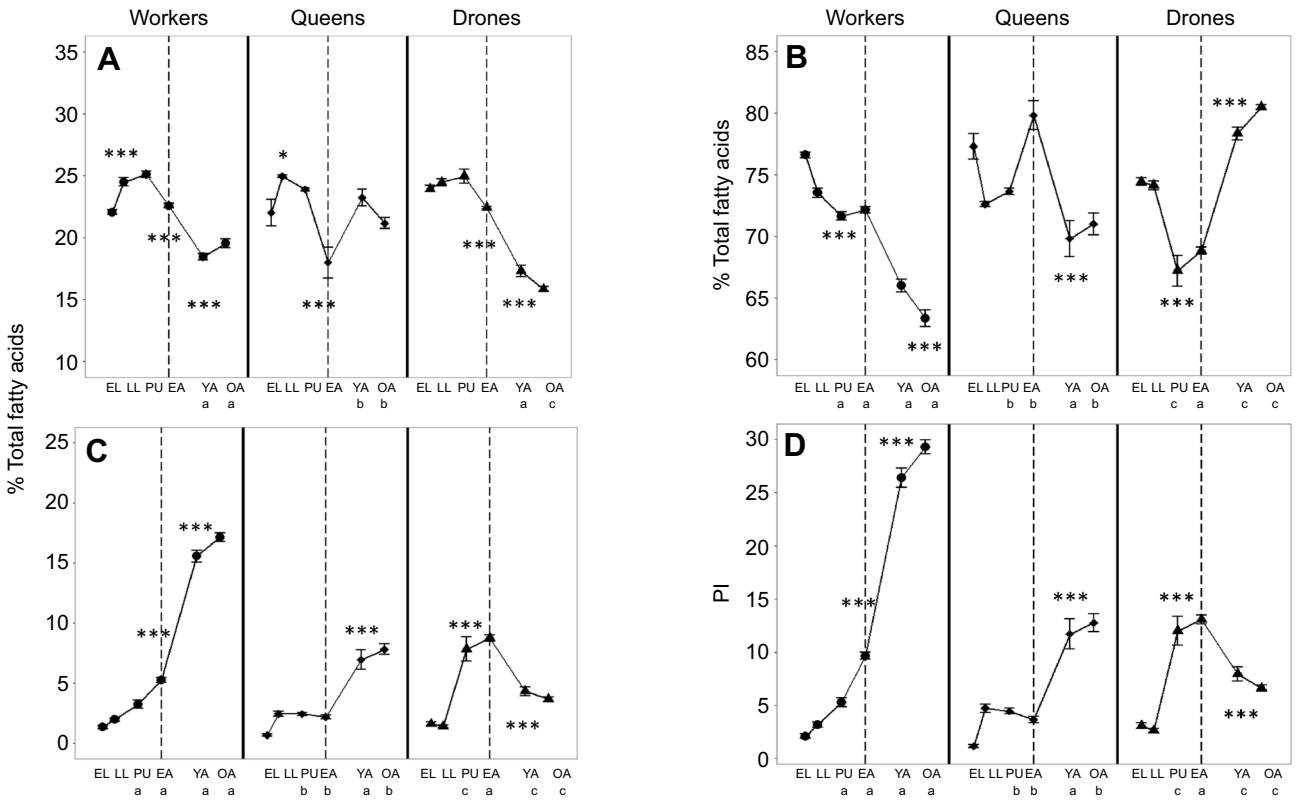

Fig. 1. Relative fatty acid content and peroxidation index of membrane phospholipid extracts of the three different castes of honey bees (*Apis mellifera*). (A) Total saturated fatty acids; (B) total monounsaturated fatty acids (MUFA); (C) total polyunsaturated fatty acids (PUFA). (D) Peroxidation index (PI) of membrane phospholipid extract. Each caste has six life-history stages (from left to right): EL, early larva; LL, late larva; PU, pupa; EA, emergent adult; YA, young adult; and OA, old adult (see Table 1 for further details on life-history stage description and Table 3 for sample size). Dashed lines indicate emergence as adult. Data are presented as means±s.e.m. and expressed as percentage of total fatty acids (mol %). Lowercase letters indicate a significant difference among the castes for a given life-history stage with $P<0.05$ (e.g. in A, young adult workers and drones differ significantly from young adult queens). Asterisks indicate a significant change from previous life-history stages within the same caste (i.e. from early larva to late larva, in workers): *$P<0.05$, **$P<0.01$, ***$P<0.001$.

whereas the decrease in MUFA in drones was limited to early development up to the pupation stage (Fig. 1B). Drones increased their MUFA levels in adulthood to become significantly higher than in both female castes. Adult queens maintained a significantly higher level of MUFA compared with workers ($P<0.001$), with MUFA levels decreasing between the early to young adult stages. Generally, queens maintained a relatively consistent MUFA level at ∼75% of total fatty acids throughout their life-history stages (Fig. 1B). Saturated fatty acids (SFA) decreased (∼25%; Fig. 1A) in both workers and drones following emergence, whereas adult queens maintained relatively stable SFA levels with a higher proportion of SFA in their membranes in adult life compared with other castes ($P<0.05$; Fig. 1A).

All bee castes maintained a very low proportion of PUFA during their larval stages (∼2% of total fatty acid; Fig. 1C). During early development, the most notable difference between the castes was a consistent increase in membrane PUFA in workers and drones compared with queens. After emergence as adults, worker bees continued to increase their level of PUFA, reaching a maximal of ∼15% of membrane fatty acids as young adults. Queens also increased their membrane PUFA level after emergence, but to a much lesser extend (7.5%). In contrast, the level of PUFA decreased in drones during adult life to ∼4% of total fatty acids.

The relative peroxidisability of membranes, measured as the PI, tended to follow the profile of changes in PUFA, being influenced primarily by the amount and type of PUFA present. All castes maintained low PI values during their larval stages of ∼2.5 (Fig. 1D). However, towards emergence, both workers and drones increased their PI values up to ∼12, with incorporation of PUFA into their membrane phospholipids. In contrast, queens consistently maintained low PI values throughout this period (Fig. 1D). At emergence, membrane PI was 2-fold higher in workers and drones than in queens ($P<0.001$). The largest increase in membrane PI was observed in workers between emergence and young adults, where membrane PI increased 2.5-fold, remaining high thereafter (at ∼27). Overall, there was a 10-fold increase in PI of workers from early larva to old adult. Queens also increased their membrane PI following emergence but to a much lesser extent, with membrane PI of 3 year old adult queens being similar to that of emergent workers (∼10). In drones, membrane PI decreased after emergence, with old drones having similar membrane PI values to adult queens (Fig. 1D).

Membrane phospholipids

All three castes showed similar changes in membrane density (nmol phospholipid mg^{-1} of bee; Fig. 2) during their development in the form of a U-shaped curve. Membrane density in early larva started high (18–21 nmol mg^{-1} of bee) before reducing abruptly in late larva and pupa stages, remaining low through to emergence. Membrane density then rose rapidly in queens and drones as adults but remained low in young adult worker bees before finally increasing in older adults (21 nmol mg^{-1} of bee) to a level similar to that found in queens and drones, and the early larval stage.

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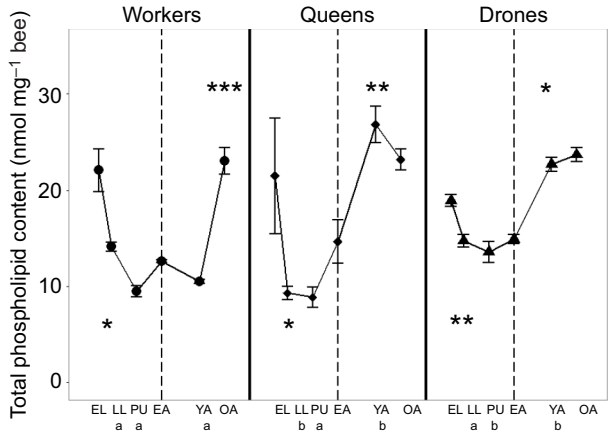

Fig. 2. Total phospholipid content in lipid extract of the three different castes of honey bees. EL, early larva; LL, late larva; PU, pupa; EA, emergent adult; YA, young adult; and OA, old adult. Data are presented as means±s.e.m. Lowercase letters indicate a significant difference among the castes for a given life-history stage. Asterisks indicate a significant change from previous life-history stages within the same caste: **P*<0.05, ***P*<0.01, ****P*<0.001. Number of replicates per group is provided in Table 3.

A quantitative comparison of the four major classes of membrane phospholipids from lipid extracts of whole bees of different castes and life stages is presented in Fig. 3. PC and PE were the main phospholipid classes found in membranes, with PIn and PS being of lower abundance. In general, the castes showed similar patterns of change during their development. It was commonly observed in all three castes that the level of PC, PE and PS in old adults was very similar to that found in early larvae. Despite numerous statistical differences, most differences between the castes remained small, except for PIn, which was expressed at much higher levels in queens as adults.

A general observation for PC was that all castes underwent similar changes during their development (Fig. 3A), with a reduction during larval development followed by an increase after emergence. The only notable difference was for the level of PC in young workers, which remained low (reflected in total phospholipids). Drones generally showed less variation in their PC level during development compared to female castes (Fig. 3A). Changes in PE levels during development were similar to those seen for total phospholipids and PC, with increases in PE delayed in young adult workers (Fig. 3B). PIn, unlike the other phospholipids, demonstrated a large amount of variability between the castes (Fig. 3C). Queens had significantly higher levels of PIn throughout life from pupal to young adult stages (from 0.5 to 3 nmol mg^{-1}; Fig. 3C). Drones showed a similar increase to queens up to emergence but then decreased their PIn level in adulthood. Workers showed low PIn levels with little change throughout development. PS levels were low compared with other phospholipids (<1 nmol mg^{-1}; see Fig. 3D) with few differences amongst the castes, normally with reductions in larva stages followed by a rise in adulthood. As previously found for both PC and PE, young adult workers maintained a PS level similar to that of emergent

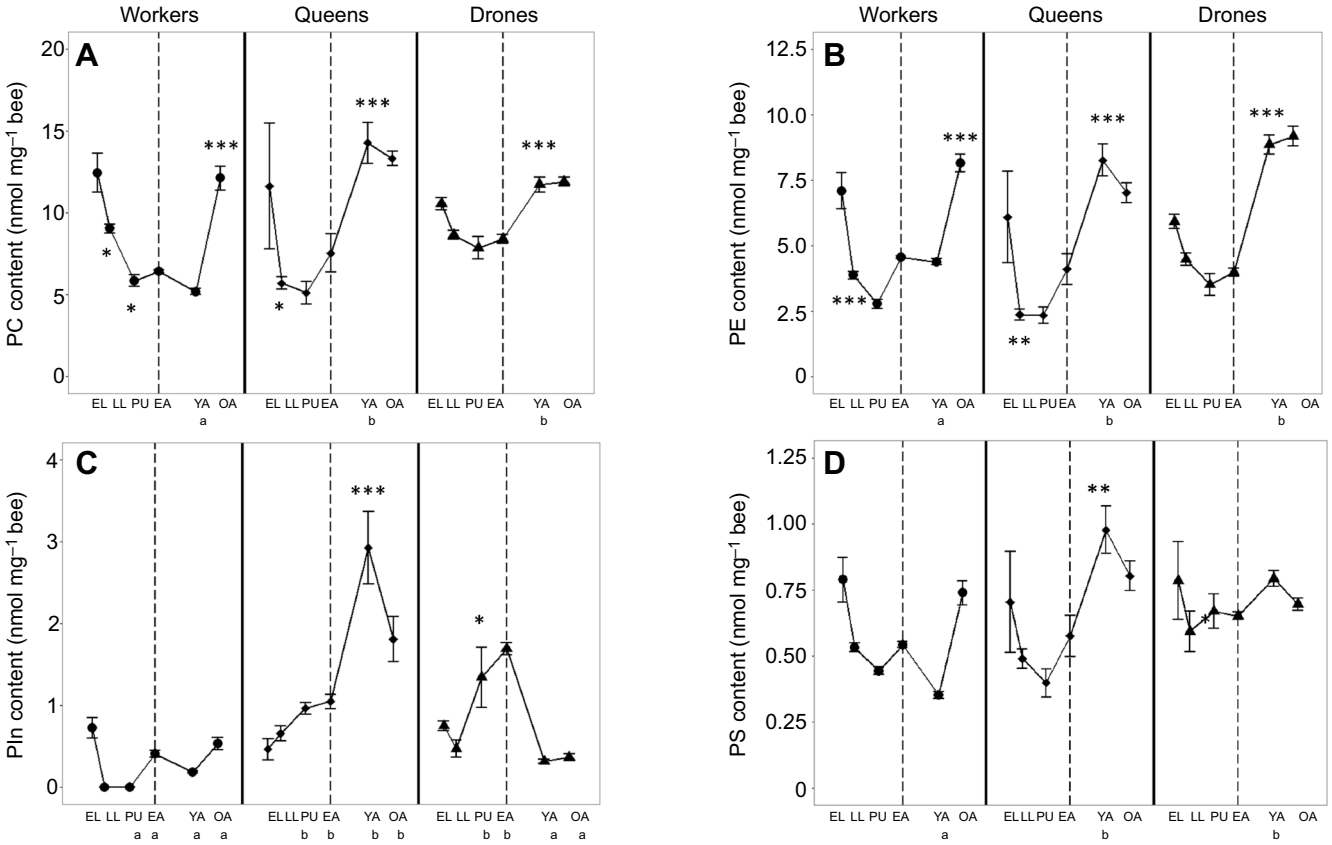

Fig. 3. Main phospholipid classes in lipid extract of the three different castes of honey bees. (A) Phosphatidylcholine (PC); (B) phosphatidylethanolamine (PE); (C) phosphatidylinositol (PIn); and (D) phosphatidylserine (PS). EL, early larva; LL, late larva; PU, pupa; EA, emergent adult; YA, young adult; and OA, old adult. Data are presented as means±s.e.m. Lowercase letters indicate a significant difference among the castes for a given life-history stage. Asterisks indicate a significant change from previous life-history stages within the same caste: **P*<0.05, ***P*<0.01, ****P*<0.001. Number of replicates per group is provided in Table 3.

adults with a delayed doubling of their PS levels in older adults, whereas queens continued to increase their PS levels from pupae to young adult (reaching the highest level of PS at ~1 nmol mg^{-1}). In contrast to the female castes, drones maintained a relatively constant level of PS throughout development and adult life.

Lysophospholipids

There were few statistical differences in the level of total lysophospholipids between castes (Table 4). Queens maintained very low levels of lysophospholipids throughout their lifetime except as early larvae. As old adults, workers and drones had higher levels of lysophospholipids compared with queens (P<0.01). Overall, the total concentration of lysophospholipids was approximately an order of magnitude lower than that of diacyl phospholipids.

Major phospholipid molecules

Molecular phospholipids with SFA and MUFA

The main phospholipid molecules detected are presented in Tables S3–S6. Phospholipid molecules that contained only SFA and MUFA (i.e. 16:0_18:1, 18:0_18:1, 16:1_18:1 and 18:1_18:1) are depicted in Table S3 (PC and PE) and Table S4 (PIn and PS). A common trend observed in abundant non-PUFA-containing phospholipid molecules for PC, PE and PS was a decrease from early larva to pupa stage followed by an increase in adult life (as observed in total phospholipids). The most abundant non-PUFA-containing phospholipid molecule in all castes was PC18:1_18:1 followed by PC16:0_18:1 (Table S3). For PE, the most abundant non-PUFA-containing combinations were PE18:1_18:1 and PE18:0_18:1 (Table S3). For PIn, non-PUFA-containing phospholipids were of extremely low abundance in workers, whereas in queens they were in higher abundance. In drones, non-PUFA-containing phospholipids were of moderate abundance, especially during adult life. In queens, the PIn molecule with the greatest abundance was PIn18:0_18:1 (Table S4). In PS, the most common non-PUFA-containing molecules were PS16:1_18:1 and PS18:1_18:1 in all castes (Table S4). In general, PS phospholipids demonstrated limited differences between the different castes.

The non-PUFA-containing phospholipid molecules within PC, PE and PS displayed very similar levels in the different castes up to emergence. However, as young adults, workers had consistently lower levels of non-PUFA-containing phospholipids (2- to 10-fold) compared with queens and drones. In old adults, these differences became diminished between the castes. Queens, particularly as adults, had a much higher level of PIn18:0_18:1 compared with adult workers and adult drones (Table S4). Queens and drones also had a higher level of PIn18:1_18:1 compared with workers during development. Overall, despite numerous statistically significant differences, the castes had similar levels of most non-PUFA-containing molecular phospholipids, with the exception of PIn18:0_18:1 in queens.

Table 4. Total lysophospholipid content of lipid extract of the three different castes of honey bees

	Lysophospholipid content (nmol mg^{-1} bee)		
Life stage	Workers	Queens	Drones
Early larva	1.0±0.3[a]	2.6±1.3[b]	0.9±0.1[a]
Late larva	0.7±0.2	0.1±0.02	0.5±0.1
Pupa	0.4±0.14	0.05±0.01	0.2±0.04***
Emergent	0.7±0.1	0.9±0.3	0.2±0.01
Young adult	0.4±0.04	0.4±0.1***	1.0±0.2***
Old adult	1.5±0.4[a,*]	0.2±0.01[b,**]	1.5±0.15[a]

Data are expressed as means±s.e.m. Lowercase letters indicate a significant difference among the castes for a given life-history stage (e.g. early larva worker versus early larva queen versus early larva drone at P<0.05). Asterisks indicate a significant change from previous life-history stages within the same caste: *P<0.05 ,**P<0.01, ***P<0.001. Number of replicates per group is provided in Table 3.

Molecular phospholipids with PUFA

The main phospholipids containing PUFA are presented in Table S5 (for PC and PE) and Table S6 (for PIn and PS). The four most abundant phospholipid combinations that contain PUFA were 16:0_18:3, 18:0_18:3, 18:1_18:2 and 18:1_18:3 for each phospholipid classes (i.e. PC, PE, PIn and PS). The level of phospholipid molecules that contained PUFA was low in all castes during development. After emergence, the castes started to differ in their level of PUFA in the phospholipid molecules. In most cases, the castes reached their maximum level of PUFA-containing phospholipids as older adults. In PC, PE and PS, old adult workers had the highest abundance of phospholipid molecules containing PUFA. However, adult queens showed a different pattern, possessing some PIn molecules with higher PUFA abundance, notably PIn18:0_18:3 (Table S6).

The level of phospholipids containing PUFA in PC and PE was at least 3-fold higher in old adult workers than in old adult queens and drones (Table S5). In adult workers and queens, PC18:1_18:3 was the most abundant PUFA-containing phospholipids whereas in drones, PC18:0_18:3 and PC18:1_18:3 were the most common but at much lower levels (Table S5). The most abundant PE phospholipids containing PUFA in all castes were PE18:0_18:3, PE18:1_18:2 and PE18:1_18:3 (Table S5). Old adult queens had a 2-fold higher level of PIn18:0_18:3 and PIn18:1_18:3 compared with both workers and drones (Table S6; P<0.05). For PS, old workers had 3 times the level of PS18:0_18:3 compared with old queens and drones (Table S6). Interestingly, the level of PUFA-containing phospholipids was similar between queens and drones as adults, for all phospholipid classes except PIn.

Plasmalogens

The level of plasmalogens for the various bee castes is presented as non-PUFA- or PUFA-containing plasmalogens as well as total plasmalogens (Table 5). For workers and queens, the total level of plasmalogens was generally low and similar during early development (i.e. early larva, late larva and pupa). After emergence, workers increased total plasmalogen levels by ~2.8-fold and queens by ~1.6-fold. Drones had a low level of total plasmalogens similar to that of the female castes at the early larva stage but soon increased plasmalogen levels to higher than those in female castes in later life stages. Drones increased plasmalogen levels ~7-fold from late larva to pupation (primarily from non-PUFA-containing plasmalogens) to the highest level of plasmalogens of any caste at any life-history stage. As adults (with few exceptions), the two female castes showed similar plasmalogen levels with ageing. The major difference between workers and queens was that the increase in total plasmalogens post-emergence was primarily due to non-PUFA plasmalogens for workers and from PUFA-containing plasmalogens for queens.

DISCUSSION

The phospholipids of each developmental and adult life-history stage (from small larva to old adults) of the two female (workers, queens) and one male caste (drones) of honey bees were examined.

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Table 5. Plasmalogen content in whole-bee extract of different life-history stages of the three castes of honey bee

		Plasmalogen content (nmol mg^{-1} bee)		
Caste	Category	Total	Non-PUFA	PUFA
Workers	Early larva	0.03±0.003^{a}	0.03±0.003^{a}	ND
	Late larva	0.01±0.002^{a,***}	0.01±0.002^{a,***}	ND
	Pupa	0.05±0.007^{a,**}	0.03±0.004^{a,**}	0.02±0.003^{a,***}
	Emergent adult	0.07±0.003^{a}	0.06±0.002^{a,***}	0.01±0.001^{a}
	Young adult	0.10±0.003^{a,**}	0.09±0.003^{a,***}	0.02±0.001^{a,**}
	Old adult	0.19±0.008^{a,**}	0.15±0.006^{a,***}	0.04±0.003^{a,*}
Queens	Early larva	0.03±0.006^{a}	0.03±0.003^{b}	ND
	Late larva	0.01±0.001^{a}	0.01±0.001^{b}	ND
	Pupa	0.02±0.002^{b}	0.01±0.002^{a,b}	ND
	Emergent adult	0.10±0.014^{a,b}	0.03±0.004^{b}	0.06±0.009^{b}
	Young adult	0.17±0.012^{a,b}	0.05±0.004^{b}	0.12±0.009^{b}
	Old adult	0.16±0.008^{a}	0.06±0.005^{b}	0.10±0.003^{b}
Drones	Early larva	0.04±0.004^{a}	0.04±0.004^{b}	ND
	Late larva	0.03±0.002^{b}	0.03±0.002^{c}	ND
	Pupa	0.22±0.133^{c,**}	0.18±0.139^{b}	0.15±0.011^{a,**}
	Emergent adult	0.13±0.004^{b}	0.04±0.002^{c}	0.08±0.004^{b}
	Young adult	0.83±0.123^{b}	0.82±0.122^{c,*}	0.02±0.003^{a}
	Old adult	1.02±0.092^{b}	1.00±0.091^{c}	0.02±0.002^{a}

Plasmalogens were compiled from all plasmalogen molecular phospholipids into three categories: non-PUFA plasmalogens that were associated only with SFA and MUFA, PUFA plasmalogens that were associated with at least one PUFA, and total plasmalogens. Data are expressed as means±s.e.m. Lowercase letters indicate a difference between the castes for the same plasmalogen category (e.g. total plasmalogens was similar between early larva workers, early larva queens and early larva drones). Level of significance was $P<0.05$ when comparing castes (e.g. early larva workers, early larva queens, early larva drones). Asterisks indicate a significant change from the previous life-history stage within the same caste (e.g. total plasmalogens changed significantly from early larva to late larva in workers): *$P<0.05$, **$P<0.01$, ***$P<0.001$. ND indicates that those phospholipids were not quantified in the extract. Number of replicates per group is provided in Table 3.

All castes were found to share a similar membrane fatty acid composition during early development, a composition that queens tended to maintain into adult life. Following emergence as adults, worker bees increased their level of PUFA in membranes at the expense of MUFA, whereas in queens and drones, the membranes remained highly monounsaturated. The increase in PUFA in the lipid of adult worker membranes occurred across most phospholipid classes, with all castes maintaining similar levels of these different classes, except for PIn, which was considerably higher in adult queens.

The membrane phospholipid fatty acid composition of honey bees was found to be relatively simple, with only six major fatty acids present i.e.16:0; 16:1, 18:0, 18:1, 18:2 and 18:3. This agrees with previous findings in honey bees (Haddad et al., 2007; Robinson and Nation, 1970; Xu and Gao, 2013) and termites (Basalingappa et al., 1972). Membrane molecular phospholipids consisted of only four to five main molecules per phospholipid class, which is similar to that of another recent study (Wegener et al., 2018). There was a lack of longer-chain (longer than 18-carbon) fatty acids in the membranes of bees. This deficit included common PUFA molecules, such as 20:4n-6 and 22:6n-3, found in marine invertebrates (Munro and Blier, 2012) and vertebrates (Abbott et al., 2010, 2012; Cortie et al., 2015; Hulbert et al., 2002). This suggests that bees lack these fatty acids in their diet and the elongase enzymes necessary to produce them *de novo*.

All bee castes had a very similar membrane fatty acid composition up to the pupation stage. As larvae, all castes had highly monounsaturated membranes (more than 70%; Fig. 1B), with very little polyunsaturation (less than 2%; Fig. 1C). However, from late larva to emergence, in both workers and drones, the level of PUFA increased, with compensatory reductions in MUFA. Queens did not display an increase in PUFA during pupation, whereas both the shorter-lived castes (i.e. workers and drones) increased PUFA in their membrane phospholipids, suggesting that the larvae of these castes were partially feeding on pollen leading up to this stage of their development (Tautz, 2008; Winston, 1987). In contrast, queens increased their level of MUFA during pupation (Fig. 1B) with a correspondent reduction in SFA in membrane phospholipids (Fig. 1A), suggesting queens are not fed pollen during their larval stage.

Following emergence, adult castes started to differ significantly in the fatty acid composition of their membrane phospholipids. Workers increased their level of PUFA during the first week of their adult life (Fig. 1C), at the expense of MUFA (Fig. 1B), whereas queens did not. This increase in PUFA was spread across most phospholipid classes, particularly PC and PE in the membranes of workers (Table S5). This change presumably reflects worker bees starting to feed on pollen even though they are also known to be fed liquid food mouth-to-mouth by older workers for the first week of adult life to complete their development (Haydak, 1970; Tautz, 2008; Winston, 1987).

As pollen has a high level of PUFA (more than 50% of total fatty acids; see Table 2), its consumption during the first week of adult life in workers led to a 3-fold increase in membrane PUFA. Thereafter, membrane phospholipids of workers remained relatively high and stable in PUFA, suggesting worker bees consistently feed on pollen throughout their adult life. Only two PUFA were found in pollen, 18:2 and 18:3, the same two PUFA found in membrane phospholipids and triglycerides (data not shown) of worker bees. In contrast, the membrane phospholipids of adult queens (genetically identical to workers) remained highly monounsaturated with very low levels of PUFA. As adults, queens are fed mouth-to-mouth by worker bees their whole life and are not normally observed eating pollen (Tautz, 2008; Winston, 1987). The low abundance of PUFA in the membrane phospholipids of queens (Haddad et al., 2007; Robinson and Nation, 1970; Xu and Gao, 2013) reflects more the fatty acid composition of royal jelly, which is very low in PUFA (Li et al., 2013; Xu and Gao, 2013).

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Membrane composition, peroxidation and ageing

PUFA have been implicated in the ageing process through their potential to oxidise and cause damage to surrounding tissues (Hulbert, 2005). The susceptibility of PUFA to oxidise (or peroxidise) is dependent upon the number of double bonds (specifically bis-allylic groups described in the Introduction). For example, 18:2 is 40 times and 18:3 is 80 times more likely to undergo peroxidation than the MUFA 18:1 (Cosgrove et al., 1987; Holman, 1954). Thus, from the relative fatty acid composition of a membrane it is possible to calculate the likelihood of lipid peroxidation (independent of other influences, e.g. antioxidant status). The PI combines the relative abundance of each PUFA multiplied by its bis-allylic methylene groups to determine a value that rates the susceptibility of a membrane to peroxidise (Hulbert, 2005). The smaller the PI value, the more resistant a membrane is to peroxidative damage and vice versa. Membrane PI inversely correlates with longevity in mammals and birds (Hulbert et al., 2007, 2017), including unique long-lived species such as the short-beaked echidna (*Tachyglossus aculeatus*, maximal lifespan of 54 years) and naked mole rat (*Heterocephalus glaber*, maximal lifespan of 32 years). Membrane PI has also been inversely correlated with the maximal lifespan in long-lived invertebrates, such as bivalve molluscs that can live up to 500 years (Munro and Blier, 2012) and different strains of *Caenorhabditis elegans* that live for different periods (Shmookler Reis et al., 2011). The same relationship has also been reported in female honey bees (Haddad et al., 2007), with the present study supporting these findings by showing that worker bees possess peroxidation-prone membranes and queens have peroxidation-resistant membranes based on membrane phospholipid composition. Therefore, membrane PUFA composition is a potential explanation for the longevity of queens.

The present study found that all castes as larva shared a similar membrane PI, but by emergence, worker bees had a significantly higher membrane PI compared with queens (Fig. 1D). This result suggests that pollen feeding occurs during the late larva–pupa stage. This aligns with the suggestion that a compound found in pollen (*P*-couramic acid) inhibits physiological processes such as ovary development in workers (Mao et al., 2015), preventing workers from becoming queens. Upon emergence, the membrane PI of workers increased a further 3-fold early on in adult life and was then maintained throughout adult life. Although queens did increase their membrane PI after emergence, this occurred to a much lesser extent, with more monounsaturated fatty acids compared with adult workers, thus making queen membranes far more resistant to peroxidation. In the present study, queens were found to maintain a low membrane PI for up to 3 years, with no significant change observed between 1 and 3 year old queens.

These results suggest that the difference between adult female bees in the potential of membranes to peroxidise is more likely to be due to events that occur after they emerged as adults. Larvae from all castes, including drones, are fed similar food early in their development. The larval food is produced by a combination of secretions from the mandibular and hypopharyngeal glands of worker bees (Haydak, 1970; Winston, 1987). The food fed to future queens (royal jelly) is an approximate 1:1 mix of hypopharyngeal: mandibular glandular secretions while the food fed to worker larvae (worker jelly) is an approximate 2:1 mix (Haydak, 1970; Winston, 1987). Royal jelly is a complex mixture consisting of amino acids, sugars, proteins and mineral salts with a low lipid content (3–10% of dry mass; Ferioli et al., 2014; Xu and Gao, 2013). The fatty composition of royal jelly is mainly composed of free ether soluble fatty acids (not triglycerides or phospholipids). Most fatty acids are SFA or MUFA of short- to medium-chain length (under 20 carbons long) that are either hydroxylated or dicarboxylic fatty acids. There is essentially no PUFA in these secretions (Li et al., 2013). One of the main lipid components of royal jelly is the fatty acid 10-hydroxy-2-decenoic acid (10 HDA), which comprises up to 6% of royal jelly (Barker et al., 1959). This particular fatty acid has been proposed to have an epigenetic role in caste determination in honey bees (Spannhoff et al., 2011). The very similar membrane PI values of the female castes as larvae suggests that the two types of jelly have the same or at least a very similar fatty acid composition. Another major difference between castes lies in the division of labour. Workers perform all tasks necessary to sustain the colony as they move through the different life-history stages (Winston, 1987). As they transition from nursing tasks to foraging, workers will increase their flight activity, and this may impact upon their longevity. However, where workers have been maintained in cages (and their flight activity is reduced), their average lifespan has been found to be between 23 to 42 days (Manning et al., 2007; Pasquale et al., 2016; Wang et al., 2014). This lifespan is very similar to that of free-living worker bees and suggests that the impact of flight activity on the longevity of worker honey bees is limited.

Contribution of phospholipids to the membrane PI of female honey bees

The major phospholipid classes containing PUFA in the female bee castes were PC, PE and PIn (Fig. 4). These phospholipids contribute relatively equally to membrane PI during larval stages and up to emergence. However, the contribution of the different phospholipid classes to PI changes significantly during adult life. Molecular phospholipids from PC and PE comprised up to 90% of the membrane PI in adult workers while PC and PE contributed to less than 50% of membrane PI in adult queens (Fig. 4). Interestingly, the castes had similar levels of PC (Fig. 3A) and PE (Fig. 3B) in adult life. Therefore, queens increase the abundance of molecular phospholipids that contain SFA and MUFA (e.g. PC/PE16:0_18:1, PC/PE18:1_18:1) during their adult life. This combination of phospholipids may inhibit peroxidation in

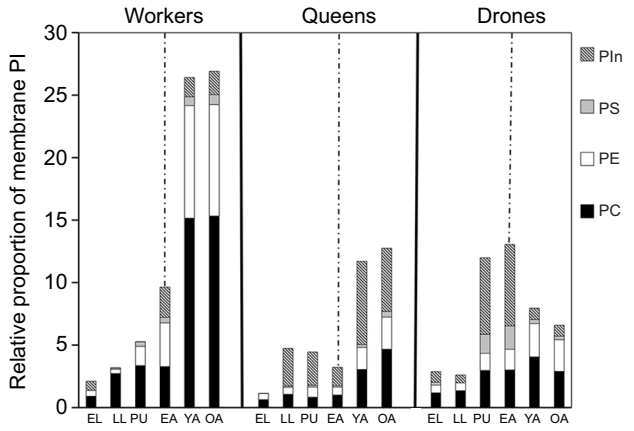

Fig. 4. Contribution of different phospholipid headgroups to the PI of membrane phospholipid extracts of the three different castes of honey bees. EL, early larva; LL, late larva; PU, pupa; EA, emergent adult; YA, young adult; and OA, old adult. Dashed lines indicate emergence as an adult. PIn, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine. Data are presented as means and expressed as a proportion of the membrane PI. Number of replicates per group is provided in Table 3.

membranes as PC16:0_18:1 has been found to delay the onset of peroxidation by increasing the lag phase of peroxidative reactions (delaying entry into the more damaging propagation phase) in experimental liposomes (Cortie and Else, 2015). This mechanism provides more time for existing antioxidants to inhibit peroxidation. Such mechanisms have yet to be explored in membrane isolated from organisms. However, a similar mechanism for reducing peroxidation has been proposed for non-methylene-interrupted PUFA found in molluscs. These extremely long-lived bivalves have peroxidation-resistant non-methylene-interrupted fatty acids, where the double bonds are separated by more than one methylene, eliminating the peroxidation sensitive bis-allylic methylene normally found on PUFA molecules (Munro and Blier, 2012).

In adult queens, PUFA in PIn phospholipid molecules contributed 50% or more to the membrane PI compared with less than 10% in adult workers (Fig. 4), with adult queens having a 4- to 6-fold higher level of PIn compared with adult workers (Fig. 3C). Adult queens had a particularly high abundance of PIn18:0_18:1 (Table S4), while workers had very little of this molecule. Such molecular phospholipids may help reduce lipid peroxidation using the same inhibition properties found for PC16:0_18:1 (Cortie and Else, 2015). Low-abundance phospholipid classes such as PIn might also be involved in lipid and cell signalling as well as membrane trafficking (Di Paolo and De Camilli, 2006). PIn phospholipids may also have potential roles in other physiological processes in social insects, such as pheromone production in queens.

Plasmalogen phospholipids have been proposed to act as reactive oxygen species scavenger molecules that can stop lipid peroxidation and confer antioxidant protection to membranes (Engelmann, 2004). Interestingly, naked mole rats with their 10-fold difference in maximal lifespan compared with normal rats have a higher abundance of plasmalogens as PE molecular phospholipids (Mitchell et al., 2007). Comparisons with invertebrates, however, are limited, with molluscs showing an inconsistent association (although only a few species were examined) between plasmalogens and longevity (Munro and Blier, 2012). In honey bees, plasmalogen levels were very similar between the three castes during development; however, following emergence, plasmalogen abundance increased in all castes to differing extents. The two female castes had a similar level of plasmalogen as adults, whereas adult drones had 10-fold higher level of plasmalogen compared with the female castes. The fatty acid composition of these plasmalogens was very different between the castes. In adult drones and adult workers, most of the plasmalogens (>80%) were associated with SFA and MUFA, whereas in adult queens, more than 60% of plasmalogens were associated with PUFA, 5-fold higher in queens compared with workers and drones (Table 5). A higher abundance of plasmalogens associated with PUFA, together with a lower level of PUFA in adult queens, suggests that queens may be using plasmalogens as a means of protecting PUFA in their membranes. Support for such a mechanism is the high level of plasmalogens associated with PUFA in emergent adult drones (Table 5). Interestingly, drones had the highest level of PUFA when emerging as adults, suggesting that drones maybe also be using plasmalogens to protect PUFA in their membrane phospholipids. However, it is important to acknowledge that the method used in the current study could not absolutely distinguish plasmalogens from isomeric alkyl-ether species by identification of the double-bond position (therefore, plasmalogen PE18:1_18:2 will be recognised as the same molecular phospholipid as PE-O18:1_18:2). The methods used may also underestimate the abundance of plasmalogens in PE by up to 30% (Abbott et al., 2013; Mitchell et al., 2007).

Queen longevity appears not to be based on increased antioxidant status as eight major antioxidant enzymes have been found to show no difference between queen and worker honey bees (Corona et al., 2005). Interestingly, the level of gene expression of antioxidant enzymes decreases with age in queens, whereas it is maintained or even increased in workers (Corona et al., 2005). In *Lasius niger* ants, queens also have lower antioxidant enzyme gene expression, as well as lower levels of enzyme activity of Cu-Zn-superoxide dismutase compared with female workers (Parker et al., 2004). This result is similar to that of another study (Hsu and Hsieh, 2014) comparing antioxidant enzymes in queen and worker bees, where activities were 2- to 10-fold higher in workers, suggesting that workers bees have a greater antioxidant capacity than queens.

Males versus females

An unexpected finding in this study was the low abundance of PUFA in membrane phospholipids of adult drones. In contrast to female workers, the relative level of PUFA decreased after emergence in the drones (Fig. 1C) to reach a level lower than that in queens. This reduction in membrane PUFA in adult drones is associated with a corresponding increase in MUFA (Fig. 1B). The low abundance of PUFA in the membrane phospholipids of drones supports the proposal that drones are fed on food other than pollen during their adult life. The literature suggests that, like worker bees, drones are fed on pollen (Sammataro and Avitabile, 1998; Tautz, 2008; Winston, 1987) but this seems not to be supported by the results showing a reduction of PUFA in membrane phospholipids of young and old adult drones compared with emergent drones. An alternative hypothesis based on the current results is that drones are dependent on workers to be fed a jelly just like queens.

The membrane fatty acid composition of adult drones produces a very low membrane PI compared with that of adult worker bees. As found in queens, drones have highly monounsaturated membranes that would make them highly resistant to lipid peroxidation. This finding seems to go against the prediction of the influence of membrane peroxidation on lifespan, given that drones are known to have a short lifespan (Rueppell et al., 2005). Adult drones also have a low amount of plasmalogens associated with PUFA (like workers; Table 5) compared with long-lived queens. The level of plasmalogens in drones increased from larva to emergence to reach levels similar to those of emergent queens. However, the level of plasmalogens associated with PUFA decreased rapidly after emergence as adults. In social insects, males are essentially a source of sperm and accomplish no other tasks within the colony. In Hymenoptera, such as honey bees, males start their sexual life with a fixed amount of sperm sufficient for one insemination because their testes start to degrade before they emerge as adults (Moors et al., 2009). Consequently, there is no clear advantage for the colony to maintain drones that cannot replenish their sperm supplies. In honey bees, as for many species of ants, males typically live for a few days as copulation is lethal and males die shortly after inseminating a queen. In most social insects, the lifespan of males is not correlated to the lifespan of queens but rather appears to be adapted to mating opportunities. For example, *Cardiocondyla* ant colonies have multiple queens and long-lived males that have a lifespan similar to that of queens (many months; Yamauchi et al., 2006). Another example of long-lived males is found in termite kings, with lifespans matching those of the long-lived queens (Korb and Thorn,

2017). In honey bees, the single queen may influence the behaviour of workers to remove drones from the hive, which will drastically shorten their lifespan. Therefore, based on their low membrane PI, adult male honey bees could be much longer lived than occurs naturally if they continued to be maintained (e.g. feed) by workers in the hive.

Conclusions

We conducted a comprehensive analysis of the phospholipidome of all three castes of honey bees at all stages of development from a free-living hive. Membrane fatty acid composition of workers and drones was similar during larval and pupation stages, with an increase in the level of PUFA in contrast to queens, which increased the level of MUFA throughout larval development and pupation. These results support the notion that workers and drones are fed a larval food that incorporates pollen, while queens are fed larval food without pollen. Following emergence as adults, workers drastically increased their PUFA levels, which led to membranes that were more susceptible to peroxidation. In contrast, the membrane phospholipids of queens remained highly monounsaturated and resistant to oxidative damage. Adult queens also had a higher abundance of plasmalogens associated with PUFA compared with short-lived workers. This difference in membrane phospholipid composition appears to be based on changes in nutrition following emergence, where workers are fed pollen, whereas queens are fed royal jelly by workers and appear not to eat pollen. Thus, the current results suggest that the extraordinary lifespan of queens may be partially explained by queens avoiding pollen consumption in preference to royal jelly. In other words, it is not what is in royal jelly but rather what the queen is avoiding (i.e. pollen) that may help to explain the difference in lifespan between the female castes. The similar membrane PI between emergent workers and old queens (3 years) suggests that emergent worker bees are an excellent model to test the causation effect of membrane PI on longevity. Drones as adults remain an enigma as they possess membranes with a low PI, suggesting that drones do not eat pollen during adult life. The short lives of drones could be explained by their dependence on workers for their maintenance and their forced removal from the hive when they are no longer required.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.M., A.J.H., P.L.E.; Formal analysis: N.M., S.H.J.B., T.W.M.; Investigation: N.M.; Data curation: N.M., G.C.B.; Writing - original draft: N.M.; Writing - review & editing: A.J.H., T.W.M., P.L.E.; Supervision: A.J.H., T.W.M., P.L.E.; Funding acquisition: T.W.M., P.L.E.

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Data availability

Data are available from the figshare digital repository: https://figshare.com/s/6c2f56de5cb6dff71922.

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.207043.supplemental

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Table S1 – Composition of internal standard added to the lipid extracts

Molecular phospholipids	nmol per sample
LPC 17:0	15
PC 19:0_19:0	80
LPE 14:0	15
PE 17:0_17:0	50
PIn 18:0_18:0	10
PS 17:0_17:0	15

LPC: Lysophosphatidylcholine; LPE: lysophosphaethanolamine; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PIn: phosphatidylinositol; PS: phosphaltidylserine. Concentrations are for post-emergent bee samples. For larvae and pupae, 50 % of the concentration values were used (e.g. 7.5 nmol for LPC 17:0).

Table S2 – List of precursor ion scans used for the lipidomics

	Ion Mode	Scan	DP	EP	CE	CXP	Mass Range	Da/ second
Phospholipid class scans								
PC	Positive	PI 184.1	100	10	40	8	640-850	200
lyso-PC	Positive	PI 184.1	100	10	40	8	490-590	200
PE	Positive	NL 141	100	10	30	8	685-950	200
Lyso-PE	Positive	NL 141	100	10	30	8	420-540	200
PS	Positive	NL 185	100	10	25	8	730-850	200
PIn	Negative	PI 241	100	10	30	8	750-1040	200
Fatty acid chain scans								
14:0	Negative	PI 227.2	100	10	55	11	580-900	1000
16:1	Negative	PI 253.2	100	10	55	11	600-900	1000
16:0	Negative	PI 255.2	100	10	55	11	600-900	1000
17:0	Negative	PI 269.3	100	10	55	11	560-900	1000
18:3	Negative	PI 277.2	100	10	40	11	600-900	1000
18:2	Negative	PI 279.2	100	10	40	11	600-900	1000
18:1	Negative	PI 281.3	100	10	55	11	600-900	1000
18:0	Negative	PI 283.3	100	10	55	11	600-900	1000
19:0	Negative	PI 297.3	100	10	55	11	600-900	1000
20:5	Negative	PI 301.2	100	10	40	11	500-1000	1000
20:4	Negative	PI 303.2	100	10	40	11	600-1000	1000
20:3	Negative	PI 305.2	100	10	40	11	600-1000	1000
20:2	Negative	PI 307.2	100	10	40	11	600-1000	1000
20:1	Negative	PI 309.2	100	10	55	11	600-1000	1000
20:0	Negative	PI 311.2	100	10	55	11	600-1000	1000
22:6	Negative	PI 327.2	100	10	40	11	700-1000	1000
22:5	Negative	PI 329.2	100	10	40	11	700-1000	1000
22:4	Negative	PI 331.2	100	10	40	11	700-1000	1000
22:3	Negative	PI 333.3	100	10	40	11	600-1000	1000
22:2	Negative	PI 335.2	100	10	40	11	700-1000	1000
22:1	Negative	PI 337.3	100	10	55	11	700-1000	1000
22:0	Negative	PI 339.3	100	10	55	11	600-1000	1000
24:1	Negative	PI 365.3	100	10	55	11	700-1000	1000
24:0	Negative	PI 367.3	100	10	55	11	700-1000	1000

Mass shifting was prevented in negative ion mode by increasing number of summed scans. *PI* precursor ion, *NL* neutral loss, *DP* declustering potential, *EP* entrance potential, *CE* collision energy, *CXP* collision cell exit potential, *PC* phosphatidylcholine; *PE* phosphatidylethanolamine; *PS* phosphatidylserine, PIn phosphatidylinositol, Lyso Lysophospholipipds.

Table S3 – Main molecular phospholipids characterised in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of bee extract. Phospholipids in this table contain only saturated (SFA) and monounsaturated fatty acid (MUFA).

		Workers	Queens	Drones			Workers	Queens	Drones
PC 16:0_18:1	Early larva	3.6 ± 0.4	3.9 ± 1.4	3.5 ± 0.2	PE 16:0_18:1	Early larva	1.1± 0.1***	1.0 ± 0.4	0.9 ± 0.03
	Late larva	3.5 ± 0.1^{a}	1.8 ± 0.1^{b}	2.8 ± 0.1^{c}***		Late larva	0.6 ± 0.02*	0.4 ± 0.02***	0.6 ± 0.03***
	Pupa	1.8 ± 0.1^{a}***	1.5 ± 0.2^{ab}	2.3 ± 0.2^{b}		Pupa	0.3 ± 0.01	0.3 ± 0.04	0.4 ± 0.04
	Emergent adult	1.5 ± 0.1^{a}	1.6 ± 0.6^{ab}	2.1 ± 0.1^{b}		Emergent adult	0.3 ± 0.01	0.3 ± 0.06	0.3 ± 0.01
	Young adult	0.6 ± 0.02^{a}***	3.1 ± 0.2^{b}	2.1 ± 0.2^{b}		Young adult	0.1 ± 0.04^{a}	0.8 ± 0.06^{b}***	0.4 ± 0.03^{c}
	Old adult	1.5 ± 0.1^{a}*	2.3 ± 0.1^{b}	1.9 ± 0.1^{b}		Old adult	0.30 ± 0.03	0.5 ± 0.04	0.4 ± 0.02
PC 18:0_18:1	Early larva	0.5 ± 0.05	0.5 ± 0.1	0.5 ± 0.03	PE 18:0_18:1	Early larva	1.9 ± 0.2	1.5 ± 0.4	1.6 ± 0.1
	Late larva	0.5 ± 0.02^{a}	0.3 ± 0.1^{b}	0.7 ± 0.04^{c}		Late larva	1.2 ± 0.1***	0.8± 0.1	1.4 ± 0.1
	Pupa	0.4 ± 0.02	0.3 ± 0.04	0.5 ± 0.07		Pupa	1.0 ± 0.04	0.8 ± 0.1	1.1 ± 0.1
	Emergent adult	0.5 ± 0.01^{a}	0.4 ± 0.05^{ab}	0.6 ± 0.02^{b}		Emergent adult	1.3 ± 0.01	1.0 ± 0.2	1.1 ± 0.05
	Young adult	0.4 ± 0.02^{a}***	0.9 ± 0.1^{b}*	0.6 ± 0.03^{c}		Young adult	0.8 ± 0.03^{a}*	1.9 ± 0.1^{b}***	1.6 ± 0.05^{b}
	Old adult	1.0 ± 0.1^{a}***	0.8 ± 0.05^{ab}	0.6 ± 0.02^{b}		Old adult	1.5 ± 0.1***	1.7 ± 0.1	1.6 ± 0.07
PC 16:1_18:1	Early larva	1.1 ± 0.1^{a}	0.7 ± 0.2^{b}	0.5 ± 0.01^{a}	PE 16:1_18:1	Early larva	0.28 ± 0.04^{a}	0.17 ± 0.05^{ab}	0.12 ± 0.003^{b}
	Late larva	0.1 ± 0.01**	0.2 ± 0.02***	0.3 ± 0.02		Late larva	0.02 ± 0.002***	0.02 ± 0.002	0.05 ± 0.004
	Pupa	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.01		Pupa	0.01 ± 0.001	0.04 ± 0.003	0.03 ± 0.003
	Emergent adult	0.3 ± 0.01	0.3 ± 0.05	0.4 ± 0.01		Emergent adult	0.05 ± 0.001	0.04 ± 0.01	0.05 ± 0.002
	Young adult	0.1 ± 0.01^{a}	1.6 ± 0.2^{b}***	1.5 ± 0.1^{b}***		Young adult	0.03± 0.002^{a}	0.34 ± 0.05^{b}***	0.27 ± 0.01^{b}***
	Old adult	0.3 ± 0.02^{a}	1.0 ± 0.1^{b}	1.5 ± 0.1^{c}		Old adult	0.07 ± 0.001^{a}	0.16 ± 0.01^{a}***	0.28 ± 0.03^{b}
PC 18:1_18:1	Early larva	6.6 ± 0.7	6.0 ± 2.0	5.6 ± 0.2	PE 18:1_18:1	Early larva	3.1 ± 0.3	2.7 ± 0.8	2.7 ± 0.1
	Late larva	4.4 ± 0.2***	3.2 ± 0.2*	4.6 ± 0.2		Late larva	1.7 ± 0.1^{a}***	0.8 ± 0.1^{b}***	2.0 ± 0.1^{a}
	Pupa	3.1 ± 0.2	3.0 ± 0.4	4.2 ± 0.3		Pupa	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
	Emergent adult	3.6 ± 0.1^{a}	5.2 ± 0.5^{b}*	4.4 ± 0.1^{b}		Emergent adult	2.0 ± 0.03**	1.7 ± 0.4	1.7 ± 0.1
	Young adult	2.3 ± 0.1^{a}	6.8 ± 0.7^{b}	6.3 ± 0.2^{b}		Young adult	2.0 ± 0.1^{a}	3.9 ± 0.3^{b}***	4.4 ± 0.3^{b}
	Old adult	5.2 ± 0.3***	7.1 ± 0.3	6.8 ± 0.2		Old adult	3.4 ± 0.1^{a}***	3.2 ± 0.2^{a}	4.6 ± 0.2^{b}

Data are expressed in nmol· mg and as mean ± s.e.m. Letter indicates significant difference among the castes for the a given life-history stage with $p < 0.05$ (e.g. levels of PC 16:0_18:1 differ significantly among all caste at Late larva stage. Asterisk indicates significant change from previous life-history stages within the same caste (i.e. level of PC 16:0_18:1 increases from Late larva to Pupa stage, in workers) with * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. Numbers of replicates per group are provided in Table 3.

Table S4 – Main molecular phospholipids characterised in phosphatidylinositol (PIn) and phosphatidylserine (PS) of bee extract. Phospholipids in this table contain only saturated (SFA) and monounsaturated fatty acid (MUFA).

		Workers	Queens	Drones
PIn 16:0_18:1	Early larva	0.14 ± 0.03[a]	0.14 ± 0.05[a]	ND
	Late larva	0.001 ± 0.0001***	ND	ND
	Pupa	0.0004 ± 0.0001	ND	ND
	Emergent adult	0.02 ± 0.002	ND	ND
	Young adult	0.005 ± 0.0004	ND	ND
	Old adult	0.03 ± 0.01	ND	ND
PIn 18:0_18:1	Early larva	0.15 ± 0.03	0.13 ± 0.03	0.20 ± 0.02
	Late larva	0.002 ± 0.0003***	0.20 ± 0.03	0.14 ± 0.03
	Pupa	0.0003 ± 0.0001[a]	0.32 ± 0.03[b]	0.25 ± 0.09[b]
	Emergent adult	0.05 ± 0.006[a]	0.48 ± 0.1[b]	0.25 ± 0.02[c]
	Young adult	0.02 ± 0.0005[a]	0.60 ± 0.1[b]	0.04 ± 0.004[a]***
	Old adult	0.04 ± 0.005[a]	0.35 ± 0.04[b]***	0.05 ± 0.008[a]
PIn 16:1_18:1	Early larva	ND	ND	ND
	Late larva	ND	ND	ND
	Pupa	ND	ND	ND
	Emergent adult	ND	ND	ND
	Young adult	ND	ND	ND
	Old adult	ND	ND	ND
PIn 18:1_18:1	Early larva	0.16 ± 0.02	0.1 ± 0.03	0.16 ± 0.01
	Late larva	0.001 ± 0.0002[a]***	0.2 ± 0.02[b]	0.07 ± 0.02[c]***
	Pupa	0.0003 ± 0.0001[a]	0.3 ± 0.01[b]***	0.05 ± 0.01[a]
	Emergent adult	0.04 ± 0.004[a]***	0.4± 0.04[b]	0.4 ± 0.01[b]***
	Young adult	0.02 ± 0.001	0.006 ± 0.001***	0.04 ± 0.003***
	Old adult	0.03 ± 0.003	0.004 ± 0.001	0.06 ± 0.01
PS 16:0_18:1	Early larva	0.12 ± 0.01[a]	0.09 ± 0.03[ab]	0.07 ± 0.01[b]
	Late larva	0.05 ± 0.002***	ND	0.07 ± 0.01
	Pupa	0.04 ± 0.002	ND	0.06 ± 0.01
	Emergent adult	0.03 ± 0.001[a]	ND	0.06 ± 0.001[b]
	Young adult	0.01 ± 0.002[a]	ND	0.05 ± 0.003[b]
	Old adult	0.03 ± 0.003	ND	0.04 ± 0.002
PS 18:0_18:1	Early larva	ND	0.010 ± 0.003	ND
	Late larva	ND	0.003 ± 0.0002	ND
	Pupa	ND	0.002 ± 0.0005[a]	0.03 ± 0.01[b]***
	Emergent adult	0.004 ± 0.0003[a]	0.004 ± 0.001[a]	0.04 ± 0.001[b]
	Young adult	0.002 ± 0.0003[a]	0.02 ± 0.003[b]	0.01 ± 0.002[ab]
	Old adult	0.003 ± 0.0005	0.01 ± 0.001	0.01 ± 0.001
PS 16:1_18:1	Early larva	0.4 ± 0.04	0.3 ± 0.08	0.3 ± 0.1
	Late larva	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.04
	Pupa	0.2 ± 0.01	0.2 ± 0.03	0.1 ± 0.1
	Emergent adult	0.3 ± 0.01	0.4 ± 0.18	0.02 ± 0.001
	Young adult	0.2 ± 0.01[a]	0.5 ± 0.04[b]	0.4 ± 0.01[ab]
	Old adult	0.3 ± 0.02	0.4 ± 0.03	0.3 ± 0.01
PS 18:1_18:1	Early larva	0.3 ± 0.03	0.3 ± 0.1	0.2 ± 0.04
	Late larva	0.2 ± 0.01[a]***	0.1 ± 0.01[a]***	0.2 ± 0.03[b]
	Pupa	0.2 ± 0.01	0.1 ± 0.02	0.1 ± 0.06
	Emergent adult	0.2 ± 0.004*	0.3 ± 0.15	0.02 ± 0.001
	Young adult	0.1 ± 0.002[a]	0.3 ± 0.03[b]	0.3 ± 0.01[b]***
	Old adult	0.2 ± 0.01[a]***	0.2 ± 0.02[a]***	0.2 ± 0.01[b]

Data are expressed in nmol· mg and as mean ± s.e.m. Letter indicates significant difference among the castes for the a given life-history stage with $p < 0.05$ (e.g. levels of PIn16:0_18:1 are similar between workers and queens at Early larva stage). Asterisk indicates significant change from previous life-history stages within the same caste (i.e. level of PIn 16:0_18:1 increases from Early larva to Late larva stage, in workers) with * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. Numbers of replicates per group are provided in Table 3.

Table S5 – Main molecular phospholipids characterised in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of bee extract. Phospholipids in this table contain at least one polyunsaturated fatty acid (PUFA).

		Workers	Queens	Drones			Workers	Queens	Drones
PC 16:0_18:3	Early larva	0.04 ± 0.005	ND	0.03 ± 0.01	PE 16:0_18:3	Early larva	ND	ND	ND
	Late larva	0.06 ± 0.005	0.02 ± 0.002	0.03 ± 0.01		Late larva	ND	ND	ND
	Pupa	0.05 ± 0.004	0.01 ± 0.001	0.06 ± 0.01		Pupa	ND	ND	0.01 ± 0.001^{b}
	Emergent adult	0.06 ± 0.003	0.02 ± 0.004	0.04 ± 0.004		Emergent adult	ND	0.002 ± 0.001^{a}	0.004 ± 0.0004^{ab}***
	Young adult	0.2 ± 0.02***	0.1 ± 0.01*	0.09 ± 0.02		Young adult	ND	0.01 ± 0.001^{a}***	0.01 ± 0.001^{b}
	Old adult	0.4 ± 0.033^{a}***	0.1 ± 0.004^{b}	ND		Old adult	ND	0.02 ± 0.002^{a}***	0.01 ± 0.001^{b}
PC 18:0_18:3	Early larva	ND	ND	ND	PE 18:0_18:3	Early larva	ND	0.02 ± 0.001	0.03 ± 0.002
	Late larva	ND	0.01 ± 0.001	ND		Late larva	ND	0.02 ± 0.002	0.02 ± 0.002
	Pupa	0.02 ± 0.001	0.01 ± 0.001	0.02 ± 0.004		Pupa	ND	0.02 ± 0.002	0.03 ± 0.005
	Emergent adult	0.04 ± 0.001	0.01 ± 0.003	0.02 ± 0.003		Emergent adult	0.1 ± 0.003	0.02 ± 0.005	0.05 ± 0.005
	Young adult	0.08 ± 0.005^{a}	0.05 ± 0.004^{a}	0.4 ± 0.04^{a}***		Young adult	0.2 ± 0.01*	0.10 ± 0.01***	0.09 ± 0.01
	Old adult	0.35 ± 0.04^{a}***	0.06 ± 0.005^{b}	0.3 ± 0.02^{a}		Old adult	0.5 ± 0.04^{a}***	0.12 ± 0.01^{b}	0.08 ± 0.005^{b}
PC 18:1_18:2	Early larva	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.002	PE 18:1_18:2	Early larva	0.20 ± 0.02^{a}	0.03 ± 0.001^{b}	0.02 ± 0.001^{b}
	Late larva	0.14 ± 0.01	0.02 ± 0.003	0.05 ± 0.01		Late larva	0.07 ± 0.01^{a}	0.003 ± 0.001^{b}	0.02 ± 0.001^{b}
	Pupa	0.10 ± 0.004	0.03 ± 0.004	0.16 ± 0.03		Pupa	0.07 ± 0.003^{a}	0.01 ± 0.001^{b}	0.03 ± 0.004^{b}
	Emergent adult	0.12 ± 0.01^{a}	0.05 ± 0.01^{a}	0.40 ± 0.05^{b}***		Emergent adult	0.04 ± 0.004^{a}	0.01 ± 0.003^{a}	0.07 ± 0.01^{b}
	Young adult	0.41 ± 0.02^{a}***	0.20 ± 0.01^{b}*	0.03 ± 0.003^{c}***		Young adult	0.16 ± 0.01^{a}***	0.07 ± 0.01^{b}***	0.10 ± 0.01^{b}
	Old adult	0.94 ± 0.06^{a}***	0.22 ± 0.02^{b}	0.04 ± 0.003^{c}		Old adult	0.25 ± 0.01^{a}***	0.08 ± 0.01b	0.10 ± 0.01^{b}
PC 18:1_18:3	Early larva	0.11 ± 0.01	0.07 ± 0.002	0.16 ± 0.01	PC 18:1_18:3	Early larva	ND	0.04 ± 0.004	0.06 ± 0.002
	Late larva	0.21 ± 0.02	0.07 ± 0.01	0.13 ± 0.01		Late larva	ND	0.02 ± 0.002^{a}	0.05 ± 0.002^{b}
	Pupa	0.14 ± 0.01	0.04 ± 0.01	0.23 ± 0.1		Pupa	ND	0.01 ± 0.002	0.04 ± 0.01
	Emergent adult	0.19 ± 0.01	0.07 ± 0.01	0.14 ± 0.01		Emergent adult	0.07 ± 0.003^{a}	0.01 ± 0.004^{b}	0.04 ± 0.003^{b}
	Young adult	0.74 ± 0.04^{a}***	0.42 ± 0.04^{b}***	0.33 ± 0.04^{b}		Young adult	0.26 ± 0.01^{a}***	0.14 ± 0.02^{b}***	0.12 ± 0.01^{b}
	Old adult	1.58 ± 0.12^{a}***	0.55 ± 0.06^{b}	0.25 ± 0.02^{c}		Old adult	0.45 ± 0.03^{a}***	0.19 ± 0.03^{b}	0.11 ± 0.01^{c}

Data are expressed in nmol· mg and as mean ± s.e.m.. Letter indicates significant difference among the castes for the a given life-history stage with $p < 0.05$ (e.g. levels of PC 16:0_18:3 differ between workers and queens at Old adult stage). Asterisk indicates significant change from previous life-history stages within the same caste (i.e. level of PC 16:0_18:3 increases from Emergent adult to Young adult stage, in workers) with * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. Numbers of replicates per group are provided in Table 3.

Table S6 – Main molecular phospholipids characterised in phosphatidylinositol (PIn) and phosphatidylserine (PS) of bee extract. Phospholipids in this table contain at least one polyunsaturated fatty acid (PUFA).

		Workers	Queens	Drones			Workers	Queens	Drones
PIn 16:0_18:3	Early larva	0.04 ± 0.1	ND	0.03 ± 0.002	PS 16:0_18:3	Early larva	ND	ND	ND
	Late larva	0.0005 ± 0.0001	0.06 ± 0.01	0.02 ± 0.004		Late larva	ND	ND	ND
	Pupa	0.0003 ± 0.0001^{a}	0.05 ± 0.004^{a}	0.12 ± 0.03^{b}***		Pupa	ND	ND	ND
	Emergent adult	0.02 ± 0.002^{a}	0.02 ± 0.003^{b}	0.10 ± 0.01^{b}		Emergent adult	ND	ND	ND
	Young adult	0.02 ± 0.002^{a}	0.2 ± 0.03^{b}***	0.02 ± 0.003^{a}***		Young adult	ND	ND	ND
	Old adult	0.03 ± 0.005^{a}	0.11 ± 0.02^{b}*	0.02 ± 0.002^{a}		Old adult	ND	ND	ND
PIn 18:0_18:3	Early larva	0.06 ± 0.01	ND	0.1 ± 0.01	PS 18:0_18:3	Early larva	ND	ND	ND
	Late larva	0.001 ± 0.0002	0.15 ± 0.02	0.06 ± 0.01		Late larva	0.01 ± 0.002	0.004 ± 0.000	ND
	Pupa	0.001 ± 0.0002	0.11 ± 0.01	0.40 ± 0.1***		Pupa	0.02 ± 0.002	0.007 ± 0.001	0.01 ± 0.004
	Emergent adult	0.20 ± 0.02^{a}***	0.10 ± 0.01^{a}	0.60 ± 0.04^{b}		Emergent adult	0.03 ± 0.001	0.005 ± 0.002	0.01 ± 0.000
	Young adult	0.09 ± 0.01^{a}	0.96 ± 0.2^{b}***	0.11 ± 0.01^{a}***		Young adult	0.04 ± 0.003	0.03 ± 0.003	0.04 ± 0.003
	Old adult	0.30 ± 0.04^{a}***	0.65 ± 0.1^{b}***	0.10 ± 0.01^{c}		Old adult	0.11 ± 0.01^{a}***	0.04 ± 0.003^{b}	0.04 ± 0.002^{b}
PIn 18:1_18:2	Early larva	ND	ND	ND	PS 18:1_18:2	Early larva	ND	ND	ND
	Late larva	ND	0.01 ± 0.001	ND		Late larva	ND	ND	ND
	Pupa	ND	0.03 ± 0.003	0.05 ± 0.02		Pupa	ND	0.001 ± 0.000	0.15 ± 0.04***
	Emergent adult	ND	0.003 ± 0.003	0.09 ± 0.01		Emergent adult	0.01 ± 0.001^{a}	0.004 ± 0.001a	0.17 ± 0.005^{b}
	Young adult	ND	0.05 ± 0.01	0.03 ± 0.003		Young adult	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.001***
	Old adult	ND	0.04 ± 0.01	0.03 ± 0.003		Old adult	0.02 ± 0.001	0.01 ± 0.000	0.01 ± 0.001
PIn 18:1_18:3	Early larva	ND	ND	0.03 ± 0.003	PS 18:1_18:3	Early larva	ND	ND	ND
	Late larva	0.004 ± 0.001	0.05 ± 0.01	0.02 ± 0.003		Late larva	0.004 ± 0.001	0.003 ± 0.001	ND
	Pupa	0.01 ± 0.001^{a}	0.05 ± 0.005^{a}	0.13 ± 0.03^{b}***		Pupa	0.01 ± 0.001	0.004 ± 0.001	0.01 ± 0.003
	Emergent adult	0.02 ± 0.0004^{a}	0.04 ± 0.003^{a}	0.16 ± 0.01^{b}		Emergent adult	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.000
	Young adult	0.02 ± 0.001^{a}	0.20 ± 0.03^{b}***	0.03 ± 0.003^{a}***		Young adult	0.02 ± 0.001	0.01 ± 0.001	0.02 ± 0.001
	Old adult	0.04 ± 0.002^{a}	0.16 ± 0.03^{b}	0.04 ± 0.005^{a}		Old adult	0.04 ± 0.002^{a}***	0.02 ± 0.003^{b}	0.02 ± 0.001^{b}

Data are expressed in nmol· mg and as mean ± s.e.m. Letter indicates significant difference among the castes for the a given life-history stage with $p < 0.05$ (e.g. levels of PIn 16:0_18:3 in workers and queens are different to drones at Pupa stage). Asterisk indicates significant change from previous life-history stages within the same caste (i.e. level of PIn 16:0_18:3 increases from Emergent adult to Young adult stage, in queens) with * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. Numbers of replicates per group are provided in Table 3.