Honeybee Caste Lipidomics in Relation to Life-History Stages and the Long Life of the Queen.
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## Summary statement

This article is investigating mechanisms that could explain the extraordinary difference in lifespan between the castes of honey bees in relation to behaviour and difference in diet.


#### 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 living 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 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 (larvae, 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 dietinduced increase in membrane PUFA results in more oxidative damage and is potentially responsible for the much shorter lifespans of worker bees compared to long-living queens.


## 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 several folds, any experimentally achieved lifespan extension (Keller and Jemielity, 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 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 $\sim 50,000$ 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 between 2-6 weeks of age. In contrast, reproductive queens remain inside the hive for all of their life except for a nuptial flight (where 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 (An age: Data base). 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 to 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 'mouth-to-mouth' a fluid by worker bees (assumed to be the same jelly they receive as larvae), 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).

PUFA's may influence longevity as they are highly oxidisable (one thousand times more likely to oxidise than MUFA; (Else and 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 bisallylic bond on the same PUFA molecule, or from surrounding PUFA molecules. This cascade produces lipid hydroperoxides, aldehydes and other byproducts 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 aging. 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 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 compares the molecular phospholipid composition of three bee castes at six different lifehistory stages from larva to old adult, from a single free-living colony of bees. It also investigates for the first time the fatty acid composition of male bees and determine what and when changes in molecular phospholipids occur in all castes.

## Material and Methods

## Source of Honey Bees and Caste Sampling

All bees were collected from the same hive at Grenfell, New South Wales (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 where they were subsequently stored at $-80^{\circ} \mathrm{C}$. Pollen (as "bee-bread") was collected from the same hive for fatty acids analysis. Total fatty acids composition of pollen (Table 2) was performed 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 ten days before being transferred into their new hive. Queens larvae were sampled at day 3 (categorize as early larva, $n=3$ ) and day 5 (categorize as late larva, $n=5$ ). After eight days, some queen cells were transferred to an incubator ( $34^{\circ} \mathrm{C}$, relative humidity of $70 \pm 5 \%$ ). Pupa queen (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 (Arline ${ }^{\circledR}$ 400XF) and monitored in natural conditions during the next 12 months. Six queens were randomly sampled for lipid characterisation in December 2015 (categorize as young queen, 12 months old). Previously marked queens of three years of age were also sampled in austral summer of $2014(n=4)$. This design reduced any genetic differences between the individuals sampled.

## 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 were very similar (Haddad et al., 2007). However, the stinger and attached venom sac of each bee were carefully removed due to 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 that is known to have a high PUFA content. Each bee was homogenized in a Geneworks® homogenization vial filled with ceramic beads using two passages of 60 sec at $6 \mathrm{~m} / \mathrm{s}$ in 10 volumes of bulk solvent mix ( $\mathrm{MeOH}+$ internal standard) repeated with MeOH 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 (see details in Table S1 in supplementary material) was added to allow for quantification of each phospholipid class. Butylated hydroxytoluene ( $0.01 \% \mathrm{w} / \mathrm{v}$ ) 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 $4^{\circ} \mathrm{C}$ in 2 mLs of methyl-tert butyl ether (MTBE), $300 \mu \mathrm{~L}$ of 150 mM of ammonium acetate (AmOAc) was added and vortexed for a further 15 min at $4^{\circ} \mathrm{C}$ (Matyash et al., 2008). Following this, the homogenate was centrifuged at $20,000 \mathrm{~g}$ for 5 min and the lipid-containg MTBE (top) phase was removed and stored under nitrogen gas at $-20^{\circ} \mathrm{C}$.

Nano-electrospray ionization 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 ${ }^{\text {TM }}$, Advion Inc., Ithaca, NY, USA). On the day of analysis, samples were diluted with $\mathrm{MeOH}: \mathrm{CHCl}_{3}(2: 1)$ containing 5 mM AmOAc to an optimal concentration of approximately $10 \mu \mathrm{M}$ of total phospholipids. Samples were loaded onto 96 -well plates, centrifuged ( $10 \mathrm{~min}, 2200 \mathrm{~g}$ ) and directly infused into the mass spectrometer. Spray parameters were optimized at a gas pressure of 0.4 psi 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 ${ }^{\text {TM }}$ (version 1.3, AB Sciex, Framingham MA, USA). Lipidview ${ }^{\mathrm{TM}}$ 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 supplementary Table S 2 . 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., PC 36:2 can be PC 18:0_18:2 or PC 18:1_18:1).

The sn-1 and sn-2 positions of each fatty acid on the molecular phospholipid species could not be identified using the current method and isomeric phospholipid species containing alkyl ethers (termed $\mathrm{O}=$ ) or vinyl ethers (plasmalogens, termed $\mathrm{P}=$ ) 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 nmol of molecular phospholipid per mg of bee ( $\mathrm{nmol} \cdot \mathrm{mg}^{-1}$ ). Phospholipid structure is reported using the nomenclature described by Liebisch et al., (Liebisch et al., 2013).

## Fatty Acid Composition

Membrane fatty acid composition, as percent 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 ${ }^{\text {TM }}$. Fatty acids are expressed as mol $\%$ of total fatty acids.

## Peroxidation Index

Membrane peroxidation index of whole bee lipid extracts were calculated as the sum of bisallylic methylene groups per 100 fatty acids according to the equation:

Peroxidation Index $(\mathrm{PI})=\left(\sum \%\right.$ di-PUFA $\left.* 1\right)+\left(\sum \%\right.$ tri-PUFA $\left.* 2\right)+\left(\sum \%\right.$ tetra-PUFA*3 $)+$ $\left(\sum \%\right.$ penta-PUFA*4) $+\left(\sum \%\right.$ hexa-PUFA*5).

## 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 between 100-296 mg as adults in the different castes (Table 3). Queen lavae 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. As adults, worker bees reach their maximum body mass as young workers ( $\sim 159 \mathrm{mg}$ ) whereas older foragers had a body mass similar to that at emergence $(\sim 100 \mathrm{mg})$. At emergence, drones possessed the largest body mass followed by queens, then workers but body mass reduced with age in drones ( $\sim 22 \%$ ) 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

Monounsaturated fatty acids (MUFA) were the dominant membrane fatty acid throughout the different life-history stages of all bee castes accounting for between $65-80 \%$ of fatty acids (Figure 1B). Worker bees continually decreased their MUFA levels throughout their development, whereas the decrease in MUFA in drones was limited to early developmental up to the pupation stage (Figure 1B). Drones increased their MUFA levels in adulthood to become significant higher compared to both female castes. Adult queens maintained a significantly higher level of MUFA compared to workers ( $p<0.001$ ) with MUFA decreasing between the early to young adult stages. Generally, queens maintained a relatively consistent MUFA level
at $\sim 75 \%$ of total fatty acids throughout their life-history stages (Figure 1B). Saturated fatty acids (SFA) decreased ( $\sim 25 \%$, Figure 1B) 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 to other castes ( $p<0.05$; Figure 1A).

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

The relative peroxidisability of membranes, measured as the peroxidation index, 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 $\sim 2.5$ (Figure 1D). However, leading to emergence both workers and drones increased their PI values up to $\sim 12$, with incorporation of PUFA into their membrane phospholipids. In contrast, queens consistently maintained low PI values throughout this period (Figure 1D). At emergence, membrane PI was 2 -fold higher in workers and drones compared to queens ( $p<0.001$ ). The largest increase in membrane PI was observed in workers between emergence and young adults where membrane PI value increased 2.5 -fold, remaining high thereafter (at $\sim 27$ ). 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 three years old adult queens being similar to that of emergent workers ( $\sim 10$ ). In drones, membrane PI decreased after emergence with old drones having similar membrane PI values to adult queens (Figure 1D).

## Membrane Phospholipids

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

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 Figure 3. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were the main phospholipid classes found in membranes, with phosphatidylinositol (PIn) and phosphatidylserine (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 PIn that 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 (Figure 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 that remained low (reflected in total phospholipids). Drones generally showed less variation in their PC level during development compared to female castes (Figure 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 (Figure 3B). PIn, unlike the other phospholipids, demonstrated a large amount of variability between the castes (Figure 3C). Queens had significantly higher levels of PIn throughout life from pupal to young adult stages (from 0.5 to $3 \mathrm{nmol} \mathrm{mg}^{-1}$, Figure 3C). Drones showed a similar increase as 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 to other phospholipids (level $<1 \mathrm{nmol} \cdot$ $\mathrm{mg}^{-1}$; see Figure 3D) with few differences amongst the castes with normally 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 emergent 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 $\sim 1 \mathrm{nmol} \mathrm{mg}^{-1}$ ). 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 adult, workers and drones have higher levels of lysophospholipids compared to queens ( $p<0.01$ ). Overall, the total concentrations of lysophospholipids were approximately an order of magnitude lower than those of diacyl phospholipids.

## Major Phospholipid Molecules

## Molecular phospholipids with SFA and MUFA

The main phospholipid molecules detected are presented in supplementary Tables S3 to S6. Phospholipid molecules that contained only saturated and monounsaturated fatty acids (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 caste (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 consistent lower levels of non-PUFA containing phospholipids ( 2 to 10 -fold) compared to 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 to adult workers and adult drones (Table S4). Queens and drones also had a higher level of PIn18:1_18:1 compared to workers during development. Overall, despite numerous statistical
significant differences, the castes had similar levels of most of non-PUFA containing molecular phospholipid species, with the exception of PIn18:0_18:1 in queens.

## Molecular phospholipids with PUFA

The main phospholipids containing PUFA are presented in supplementary Table S5 (for PC and PE) and supplementary 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; 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, PC 18:1_18:3 was the most abundant PUFA containing phospholipids whereas in drones PC 18:0_18:3 and PC 18:1_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 have a 2 -fold higher level of PIn18:0_18:3 and PIn18:1_18:3 compared to both workers and drones (Table S6; $p<0.05$ ). For PS, old workers had 3 times the level of PS18:0_18:3 compared to 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 $\sim 2.8$ fold and queens, by $\sim 1.6$ fold. Drones had a low level of total plasmalogens similar to that of the female castes at the early larvae stage but soon increased plasmalogen levels being higher than female castes in later life stages. Drones increased plasmalogen levels from late larva to
pupation $\sim 7$-fold (primarily from non-PUFA containing plasmalogens) to possess 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 made up from 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. 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 polyunsaturated fatty acids (PUFA) in membranes at the expense of monounsaturated fatty acids (MUFA), whereas queens and drones 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 phosphatidylinositol (PIn) that 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 only consisted of four to five main molecules per phospholipid classes which is similar to that of another recent study (Wegener et al., 2018). There was a lack of longerchained (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; Abbott et al., 2012; Cortie et al., 2015; Hulbert et al., 2002). This suggests 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, the castes were highly monounsaturated (more than 70\%, Figure 1B), with very little polyunsaturation (less than $2 \%$, Figure 1C). However, from late-larvae 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 (Figure 1B) with a correspondent reduction in SFA in membrane phospholipids (Figure 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 (Figure 1C), at the expenses of MUFA (Figure 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).

Since 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 i.e. being very low in PUFA (Li et al., 2013; Xu and Gao, 2013).

## 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 their number of double bonds (specifically bis-allylic groups described in the introduction). For example, 18:2 are $40 x$ and 18:3 80x times more likely to undergo peroxidation than the MUFA 18:1 (Cosgrove et al., 1987; Holman, 1954). Thus, knowing 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 peroxidation index (PI) combines the relative amount 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; Hulbert et al., 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 peroxidation index 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 showing worker bees possess peroxidation-prone and queens 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 peroxidation index, but by emergence, worker bees had a significantly higher membrane peroxidation index compared to queens (Figure 1D). This result suggests that pollen feeding occurs during the late larval - pupa stage. This aligns with the suggestion that a compound found in pollen ( $p$ couramic acid), inhibit physiological processes such as ovary development in workers (Mao et al., 2015) preventing workers from becoming queens. Upon emergence, the membrane peroxidation index of workers increases a further 3-fold early on in adult life and is then maintained throughout adult life. Although queens do increase their membrane peroxidation index after emergence, this occurs to a much less extent with more monounsaturated compared to adult workers and thus queen membranes are far more resistant to peroxidation. In the
present study, queens were found to maintain a low membrane PI for up to three years, with no significant change observed between one and three year old queens.

These results suggest that the difference in the potential of membranes to peroxidise between adult female bees 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 mix of 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-carbon long) that are either hydroxylated in terminal or intermediate position or as 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- $\Delta_{2}$-decenoic Acid ( 10 HAD ), which composes up to $6 \%$ of royal jelly (Barker et al., 1959). This particular fatty acid has been proposed to have an epigenetic role involved in caste determination in honey bees (Spannhoff et al., 2011). The very similar membrane peroxidation index values of the female castes as larvae suggests that both 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 labor. Workers perform all tasks necessary to sustain the colony as they transit through to different life-history stages (Winston, 1987). As they transit from nursing tasks to foragers, 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 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 peroxidation index of female honey bees

The major phospholipid classes containing PUFA in the female bee castes were phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PIn) (Figure 4). These phospholipids contribute relatively equally to membrane peroxidation index during larval stages and up to emergence. However, the contribution of the different phospholipid classes to peroxidation index change significantly during adult life. Molecular phospholipids from PC and PE made up to $90 \%$ of the membrane peroxidation index in adult workers while PC and PE contributed to less than $50 \%$ of membrane peroxidation index in adult queens (Figure 4). Interestingly, the castes had similar levels of PC (Figure 2A) and PE (Figure 2B) in adult life. Therefore, queens increase the abundance of molecular phospholipids that contain SFA and MUFA (e.g. PC/PE 16:0_18:1; PC/PE 18:1_18:1) during their adult development. This combination of phospholipids may inhibit peroxidation in membranes as PC 16:0_18:1 has been found to delay the onset of peroxidation by increasing the lag phase of peroxidative reactions (delay 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 animals. However, a similar mechanism for reducing peroxidation has been proposed for non-methylene interrupted PUFA found in molluscs. These extremely long-living bivalves have peroxidation resistant non-methylene interrupted fatty acids, where the double bonds are separated by more than one methylene eliminating the peroxidation sensitive bisallylic 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 peroxidation index compared to less than $10 \%$ in adult workers (Figure 4) with adult queens having 4 to 6 -fold higher level of PIn compare to adult workers (Figure 3C). Adult queens had particularly high abundance of PIn 18:0_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 PC 16:0_18:1 (Cortie and Else, 2015). Low abundant phospholipid classes such as PIn might also be involved in lipid and cell signaling 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 ROS 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 to normal rats have a higher abundance of plasmalogens as PE molecular phospholipids (Mitchell et al., 2007). Comparisons on 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. Both females had a similar level of plasmalogen as adults while adult drones had 10-fold higher level of plasmalogen compared to 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 to SFA and MUFA whereas in adult queens, more than $60 \%$ of plasmalogens were associated with PUFA, 5 -fold higher in queens compared to 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 mechanism is the high level of plasmalogens associated with PUFA in emergent drones (Table 5). Interestingly, drones had the highest level of PUFA when emerging as adults, suggesting that drones maybe also 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 PE-18:1_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 ants, queens (Lasius niger) also have lower antioxidant enzyme gene expression, as well as lower levels of enzyme activity of $\mathrm{Cu}-\mathrm{Zn}$-superoxide dismutase compared to 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.

## Male vs. 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 male drones (Figure 1C) to reach a level lower than queens. This reduction in membrane PUFA in adult drones is associated with a corresponding increase in MUFA (Figure 1B). The low abundance of PUFA in membrane phospholipids of drones support the proposal that drones are fed on food other than pollen during their adult life. Literature suggests that drones are feed on pollen like worker bees (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 adult drones compared to 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 peroxidation index value compared to 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 the 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 to long-living 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 decreases 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 honeybees, 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 ants colonies have multiple queens and long-living males that have a lifespan similar to those of queens (many months; Yamauchi et al., 2006). Another
example of long-living males is found in termite kings, with lifespans matching long-living 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 peroxidation index, 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

A comprehensive analysis of the phospholipidome of all three castes of honey bees at all stages of development from a free-living hive has been conducted. Membrane fatty acid composition of workers and drones was similar during larval and pupation stages with an increase in level of PUFA in contrast to queens that increased the level of MUFA throughout larval development and pupation. Those results support the notion that 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 increased drastically their levels of PUFA which lead to membranes that are more susceptible to peroxidation. In contrast, the membrane phospholipids of queens remain highly monounsaturated and resistant to oxidative damage. Adult queens also had a higher abundance of plasmalogens associated with PUFA compared to short-living workers. This difference in membrane phospholipid composition appears to be based on changes in nutrition following emergence where workers are fed on 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 peroxidation index between emergent workers and old queens (3 years) suggests that emergent worker bees are an excellent model to test the causation effect of membrane peroxidation index on longevity. Drones as adults remain an enigma as they possess membranes with a low peroxidative index 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.

## Authors individual contributions

N. Martin.; Conceptualization, Data curation, Formal analysis, wrote original draft
A. J. Hulbert: Conceptualization, Supervision, review and editing
G. C. Brenner: Data curation
S. H. J. Brown: Formal analysis
T. W. Mitchell: Formal analysis, Supervision, review and editing
P. L. Else: Conceptualization, Supervision, review and editing

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## Competing interest

No competing interests declared

## References:

Abbott, S. K., Else, P. L. and Hulbert, A. J. (2010). Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 PUFA. Br J Nutr 103, 522529.

Abbott, S. K., Else, P. L., Atkins, T. A. and Hulbert, A. J. (2012). Fatty acid composition of membrane bilayers: Importance of diet polyunsaturated fat balance. Biochim. Biophys. Acta BBA Biomembr. 1818, 1309-17.

Abbott, S. K., Jenner, A. M., Mitchell, T. W., Brown, S. H. J., Halliday, G. M. and Garner, B. (2013). An improved high-throughput lipid extraction method for the analysis of human brain lipids. Lipids 48, 307-318.

An age: Data base An age: the animal ageing and longevity database.
Barker, S. A., Foster, A. B., Lamb, D. C. and Hodgson, N. (1959). Identification of 10-hydroxy- $\Delta 2$ decenoic acid in royal jelly. Nature 183, 996.

Basalingappa, S., Badami, R. C. and Kudari, S. M. (1972). The gross fatty acid composition of the body fat from the termite (Odontotermes assmuthi) queen. Biochem. J. 128, 44P-45P.

Beckman, K. B. and Ames, B. N. (1998). The free radical theory of aging matures. Physiol. Rev. 78, 547-581.

Corona, M., Hughes, K. A., Weaver, D. B. and Robinson, G. E. (2005). Gene expression patterns associated with queen honey bee longevity. Mech Ageing Dev 126, 1230-1238.

Cortie, C. and Else, P. (2015). An antioxidant-like action for non-peroxidisable phospholipids using ferrous iron as a peroxidation initiator. Fac. Sci. Med. Health - Pap. 1303-1307.

Cortie, C. H., Hulbert, A. J., Hancock, S. E., Mitchell, T. W., McAndrew, D. and Else, P. L. (2015). Of mice, pigs and humans: An analysis of mitochondrial phospholipids from mammals with very different maximal lifespans. Exp. Gerontol. 70, 135-143.

Cosgrove, J. P., Church, D. F. and Pryor, W. A. (1987). The kinetics of the autoxidation of polyunsaturated fatty acids. Lipids 22, 299-304.

Di Paolo, G. and De Camilli, P. (2006). Phosphoinositides in cell regulation and membrane dynamics. Nature 443, 651-657.

Else, P. L. and Kraffe, E. (2015). Docosahexaenoic and arachidonic acid peroxidation: it 's a within molecule cascade. Biochim. Biophys. Acta BBA - Biomembr. 1848, 417-421.

Engelmann, B. (2004). Plasmalogens: targets for oxidants and major lipophilic antioxidants. Biochem. Soc. Trans. 32, 147-150.

Faulks, S. C., Turner, N., Else, P. L. and Hulbert, A. J. (2006). Calorie restriction in mice: effects on body composition, daily activity, metabolic rate, mitochondrial reactive oxygen species production, and membrane fatty acid composition. J. Gerontol. A. Biol. Sci. Med. Sci. 61, 781-794.

Ferioli, F., Armaforte, E. and Caboni, M. F. (2014). Comparison of the lipid content, fatty acid profile and sterol composition in local italian and commercial royal jelly samples. J. Am. Oil Chem. Soc. 91, 875-884.

Haddad, L. S., Kelbert, L. and Hulbert, A. J. (2007). Extended longevity of queen honey bees compared to workers is associated with peroxidation-resistant membranes. Exp. Gerontol. 42, 601-609.

Halliwell, B. and Gutteridge, J. M. C. (2007). Free radicals in biology and medicines. 4th ed. Oxford University Press.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298-300.

Haydak, M. H. (1970). Honey bee nutrition. Annu. Rev. Entomol. 15, 143-156.
Holman, R. T. (1954). Autoxidation of fats and related substances. Prog. Chem. Fats Other Lipids 2, 51-98.

Hsieh, Y.-S. and Hsu, C.-Y. (2013). Oxidative stress and anti-oxidant enzyme activities in the trophocytes and fat cells of queen honeybees (Apis mellifera). Rejuvenation Res 16, 295303.

Hsu, C.-Y. and Hsieh, Y.-S. (2014). Oxidative stress decreases in the trophocytes and fat cells of worker honeybees during aging. Biogerontology 15, 129-137.

Hulbert, A. J. (2005). On the importance of fatty acid composition of membranes for aging. J. Theor. Biol. 234, 277-288.

Hulbert, A. J., Rana, T. and Couture, P. (2002). The acyl composition of mammalian phospholipids: an allometric analysis. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 132, 515-527.

Hulbert, A. J., Faulks, S. C., Harper, J. M., Miller, R. A. and Buffenstein, R. (2006). Extended longevity of wild-derived mice is associated with peroxidation-resistant membranes. Mech. Ageing Dev. 127, 653-657.

Hulbert, A. J., Pamplona, R., Buffenstein, R. and Buttemer, W. A. (2007). Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87, 1175-1213.

Hulbert, A. J., Martin, N. and Else, P. L. (2017). Lipid peroxidation and animal longevity. In Lipid Peroxidation: Inhibition, Effects and Mechanisms, .

Keller, L. and Jemielity, S. (2006). Social insects as a model to study the molecular basis of ageing. Exp. Gerontol. 41, 553-556.

Korb, J. and Thorn, B. (2017). Sociality in termites. In Comparative Social Evolution, pp. 84-123. Cambridge University Press.

Li, X., Huang, C. and Xue, Y. (2013). Contribution of lipids in honeybee (Apis mellifera) royal jelly to health. J. Med. Food 16, 96-102.

Liebisch, G., Vizcaíno, J. A., Köfeler, H., Trötzmüller, M., Griffiths, W. J., Schmitz, G., Spener, F. and Wakelam, M. J. O. (2013). Shorthand notation for lipid structures derived from mass spectrometry. J. Lipid Res. 54, 1523-1530.

Lucas, E. R. and Keller, L. (2014). Ageing and somatic maintenance in social insects. Curr. Opin. Insect Sci. 5, 31-36.

Manning, R. and Harvey, M. (2002). Fatty acids in honeybee-collected pollens from six endemic Western Australian eucalypts and the possible significance to the Western Australian beekeeping industry. Aust. J. Exp. Agric. 42, 217-223.

Manning, R., Rutkay, A., Eaton, L. and Dell, B. (2007). Lipid-enhanced pollen and lipid-reduced flour diets and their effect on the longevity of honey bees (Apis mellifera L.). Aust. J. Entomol. 46, 251257.

Mao, W., Schuler, M. A. and Berenbaum, M. R. (2015). A dietary phytochemical alters casteassociated gene expression in honey bees. Sci. Adv. 1, e1500795.

Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A. and Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J. Lipid Res. 49, 11371146.

Mitchell, T. W., Buffenstein, R. and Hulbert, A. J. (2007). Membrane phospholipid composition may contribute to exceptional longevity of the naked mole-rat (Heterocephalus glaber): A comparative study using shotgun lipidomics. Exp. Gerontol. 42, 1053-1062.

Moors, L., Schoeters, E., Coudron, K. and Billen, J. (2009). Morphological changes in the male accessory glands and testes in Vespula vulgaris (Hymenoptera, Vespidae) during sexual maturation. Invertebr. Biol. 128, 364-371.

Munro, D. and Blier, P. U. (2012). The extreme longevity of Arctica islandica is associated with increased peroxidation resistance in mitochondrial membranes. Aging Cell 11, 845-855.

Norris, S. E., Friedrich, M. G., Mitchell, T. W., Truscott, R. J. W. and Else, P. L. (2015). Human prefrontal cortex phospholipids containing docosahexaenoic acid increase during normal adult aging, whereas those containing arachidonic acid decrease. Neurobiol. Aging 36, 16591669.

Page, R. E. and Peng, C. Y. S. (2001). Aging and development in social insects with emphasis on the honey bee, Apis mellifera L. Exp. Gerontol. 36, 695-711.

Parker, J. D., Parker, K. M., Sohal, B. H., Sohal, R. S. and Keller, L. (2004). Decreased expression of Cu-Zn superoxide dismutase 1 in ants with extreme lifespan. PNAS 101, 3486-3489.

Pasquale, G. D., Alaux, C., Conte, Y. L., Odoux, J.-F., Pioz, M., Vaissière, B. E., Belzunces, L. P. and Decourtye, A. (2016). Variations in the Availability of Pollen Resources Affect Honey Bee Health. PLOS ONE 11, e0162818.

Robinson, F. and Nation, J. (1970). Long chain fatty acid in honeybees in relation to sex, caste, and food during development. J. Apircultural Res. 9, 121-127.

Rueppell, O., Fondrk, M. K. and Page, R. E. (2005). Biodemographic analysis of male honey bee mortality. Aging Cell 4, 13-19.

Sammataro, D. and Avitabile, A. (1998). The beekeeper's handbook. Cornell University Press.

Shmookler Reis, R. J., Xu, L., Lee, H., Chae, M., Thaden, J. J., Bharill, P., Tazearslan, C., Siegel, E., Alla, R., Zimniak, P., et al. (2011). Modulation of lipid biosynthesis contributes to stress resistance and longevity of C. elegans mutants. Aging 3, 125-147.

Spannhoff, A., Kim, Y. K., Raynal, N. J.-M., Gharibyan, V., Su, M.-B., Zhou, Y.-Y., Li, J., Castellano, S., Sbardella, G., Issa, J.-P. J., et al. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. EMBO Rep. 12, 238-243.

Tautz, J. (2008). The buzz about bees: biology of a superorganism. Springer Science \& Business Media.

Wang, H., Zhang, S.-W., Zeng, Z.-J. and Yan, W.-Y. (2014). Nutrition affects longevity and gene expression in honey bee (Apis mellifera) workers. Apidologie 45, 618-625.

Wang, Y., Ma, L.-T. and Xu, B.-H. (2015). Diversity in life history of queen and worker honey bees, Apis mellifera L. J. Asia-Pac. Entomol. 18, 145-149.

Wegener, J., Jakop, U., Schiller, J. and Müller, K. (2018). The membrane phospholipid composition of honeybee (Apis mellifera) workers reflects their nutrition, fertility, and vitellogenin stores. Insectes Sociaux 1-11.

Winston, M. L. (1987). The biology of the honey bee. Harvard University Press.
Xu, X. and Gao, Y. (2013). Isolation and characterization of proteins and lipids from honeybee (Apis mellifera L.) queen larvae and royal jelly. Food Res. Int. 54, 330-337.

Yamauchi, K., Ishida, Y., Hashim, R. and Heinze, J. (2006). Queen-queen competition by precocious male production in multiqueen ant colonies. Curr. Biol. 16, 2424-2427.

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

| Stages | Description |
| :--- | :--- |
| Early larva | small size larva |
| Late larva | big size larva |
| Pupa | cell capped, bee with soft exoskeleton, <br> hairless, eyes developed, no wing developed |
| Emergent | collected after emerging from the capped <br> cell, still with soft exoskeleton, no contact <br> with other bee, haven't flown yet; |
| Young adult workers | hair fully developed, collected in the hive <br> doing nursing related tasks, without any <br> pollen on their legs; <br> collected near the entrance of the hive, carry <br> pollen, wings still intact; dark colour |
| Young adult queens | Marked after emerging and sampled at 12 <br> months |
| Old adult queens | Marked after emerging and sampled at 3 <br> years |
| Young adult drones | Hair fully developed and collected in the <br> hive, wings still intact, bright colour, large <br> eyes and larger bodies compare to workers <br> Collected inside the hive, wings wearing-out, <br> dark colour, large eyes and larger bodies <br> compare to workers |
| Old adult drones | cors |

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.

Table 2 - Relative proportion of total fatty acid composition of "bee bread" pollen.

| Fatty acids | \% of total fatty acids |
| :--- | ---: |
| $14: 0$ | $4.2 \pm 0.4$ |
| $16: 0$ | $21.5 \pm 0.9$ |
| $16: 1$ | $0.9 \pm 0.7$ |
| $17: 0$ | $6.8 \pm 0.6$ |
| $18: 0$ | $2.9 \pm 0.3$ |
| $18: 1$ | $5.2 \pm 2.1$ |
| $18: 2$ | $28.9 \pm 1.2$ |
| $18: 3$ | $28.7 \pm 1.0$ |
| $20: 0$ | Trace |
| $20: 1$ | $0.8 \pm 0.6$ |
| Total SFA | $35.5 \pm 1.5$ |
| Total MUFA | $6.9 \pm 2.9$ |
| Total PUFA | $57.7 \pm 1.4$ |
| Peroxidation index | $86.4 \pm 2.2$ |

Total lipid fatty acids, $n=3$. Data are expressed in mol $\%$ and as mean $\pm$ s.e.m

Table 3 - Body mass (mg) of different bee castes

| Life stage | $\boldsymbol{n}$ | Workers | $\boldsymbol{n}$ | Queens | $\boldsymbol{n}$ | Drones |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Early larva | 10 | $1.6 \pm 0.2^{\mathrm{a}}$ | 3 | $16.4 \pm 6.1^{\mathrm{b}}$ | 9 | $21.9 \pm 1.7^{\mathrm{b}}$ |
| Late larva | 10 | $76.5 \pm 4.9^{\mathrm{a}}$ | 5 | $207.6 \pm 35.6^{\mathrm{b}}$ | 10 | $85.7 \pm 12.7^{\mathrm{a}}$ |
| Pupa | 10 | $106.3 \pm 5.3^{\mathrm{a}}$ | 5 | $255.2 \pm 5.2^{\mathrm{b}}$ | 7 | $295.7 \pm 17.0^{\mathrm{b}}$ |
| Emergent | 9 | $105.2 \pm 1.0^{\mathrm{a}}$ | 5 | $183.3 \pm 7.3^{\mathrm{b}}$ | 5 | $269.0 \pm 6.2^{\mathrm{c}}$ |
| Young Adult | 10 | $158.7 \pm 5.7^{\mathrm{a}}$ | 6 | $283.6 \pm 6.7^{\mathrm{b}}$ | 8 | $210.5 \pm 6.7^{\mathrm{c}}$ |
| Old Adult | 10 | $100.0 \pm 4.0^{\mathrm{a}}$ | 4 | $255.2 \pm 6.8^{\mathrm{b}}$ | 10 | $207.4 \pm 1.8^{\mathrm{c}}$ |

Young adult queens were 12 months old. Old adult queens were 3 years old. Data are presented as mean $\pm$ s.e.m. and expressed in milligram (mg). Letter ( $\mathrm{a}, \mathrm{b}, \mathrm{c}$ ) indicates 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 compare to 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).

Table 4 - Amount of total lysophospholipids in lipid extract of the three different castes of honey bees (Apis mellifera).

| Life stages | Workers | Queens | Drones |
| :--- | :--- | :--- | :--- |
| Early larva | $1.0 \pm 0.3^{\mathrm{a}}$ | $2.6 \pm 1.3^{\mathrm{b}}$ | $0.9 \pm 0.1^{\mathrm{a}}$ |
| Late larva | $0.7 \pm 0.2$ | $0.1 \pm 0.02$ | $0.5 \pm 0.1$ |
| Pupa | $0.4 \pm 0.14$ | $0.05 \pm 0.01$ | $0.2 \pm 0.04^{* * *}$ |
| Emergent | $0.7 \pm 0.1$ | $0.9 \pm 0.3$ | $0.2 \pm 0.01$ |
| Young | $0.4 \pm 0.04$ | $0.4 \pm 0.1^{* * *}$ | $1.0 \pm 0.2^{* * *}$ |
| Old | $1.5 \pm 0.4^{\mathrm{a} *}$ | $0.2 \pm 0.01^{\mathrm{b} * *}$ | $1.5 \pm 0.15^{\mathrm{a}}$ |

Data are presented as mean $\pm$ s.e.m. and expressed as $\mathrm{nmol} \cdot \mathrm{mg}^{-1}$ of bee. Letter indicates significant difference among the castes for a given life-history stage (e.g. early larva worker vs early larva queen vs early larva drone at $p<0.05)$. Asterisk indicates significant change from previous life-history stages within the same caste with $* p<$ $0.05,{ }^{* *} p<0.01,{ }^{* * *} p<0.001$. Numbers of replicates per group are provided in Table 3.

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

| Caste | Category | Plasmalogens <br> Total | Plasmalogens <br> Non PUFA | Plasmalogens <br> PUFA |
| :--- | :--- | :--- | :--- | :--- |
| Workers | Parly larva | $0.03 \pm 0.003^{\mathrm{a}}$ | $0.03 \pm 0.003^{\mathrm{a}}$ | ND |
|  | Late larva | $0.01 \pm 0.002^{\mathrm{a} * * *}$ | $0.01 \pm 0.002^{\mathrm{a} * * *}$ | ND |
|  | Emergent adult | $0.07 \pm 0.003 \mathrm{a}$ | $0.06 \pm 0.002^{\mathrm{a} * * *}$ | $0.01 \pm 0.001^{\mathrm{a}}$ |
|  | Young adult | $0.10 \pm 0.003^{\mathrm{a} * *}$ | $0.09 \pm 0.003^{\mathrm{a} * * *}$ | $0.02 \pm 0.001^{\mathrm{a} * *}$ |

Plasmalogens were compiled from all plasmalogens molecule phospholipid species into three categories: Plasmalogen Non PUFA that were associated only to saturated (SFA) and monounsaturated (MUFA) fatty acids, plasmalogens PUFA that were associated to at least one PUFA and total. Data are presented as mean $\pm$ s.e.m. and expressed as $\mathrm{nmol} \cdot \mathrm{mg}^{-1}$ of bee. Letter (a, $\mathrm{b}, \mathrm{c}$ ) indicates a difference between the castes for the same plasmalogens category (e.g. Total plasmalogens was similar between early larva workers, early larva queens and early larva drones). Level of significance was minimal $\mathrm{p}<0.05$ when comparing castes (e.g. early larva workers, early larva queens, early larva drones). Asterix indicates 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) $* \mathrm{p}<0.05, * * \mathrm{p}<0.01, * * * \mathrm{p}<0.001$. ND indicates that those phospholipids were not quantified in the extract. Numbers of replicates per group are provided in Table 3.

Figures


Workers
Queens
Drones



Figure 1 - Relative proportion of saturated fatty acids (SFA; A), monounsaturated (MUFA; B) and polyunsaturated (PUFA; C) in membrane phospholipid extracts of the three different castes of honey bees (Apis mellifera). Peroxidation index (PI) of membrane phospholipid extract is presented in D. Each caste has six life-history stages described as (from left to right): EL: early larva, LL: late larva, PU: pupa, EA: emergent adult, YA young adult and OA: old adults (see Table 1 for further details on life-history stage description and Table 1 for sample size). Dash line indicate emergence as adult. Data are presented as mean $\pm$ s.e.m. and expressed as percent of total fatty acids (mol \%). Letter on x axis indicates significant difference among the castes for a given life-history stage with $p<0.05$ (e.g. in A, Young adult worker and drones differ significantly to young adult queens). Asterisk indicates significant change from previous life-history stages within the same caste (i.e. from early larva to late larva, in workers) with $* p<0.05,{ }^{* *} p<0.01, * * * p<0.001$.


Figure 2- Amount of total phospholipids in lipid extract of the three different castes of honey bees (Apis mellifera). Each caste has six life-history stages characterised. Stages are described as (from left to right): early larva (EL), late larva (LL), pupa (PU), emergent adult (EA), young adult (YA) and old adult (OA) bees. Data are presented as mean $\pm$ s.e.m. and expressed as $\mathrm{nmol} \cdot \mathrm{mg}^{-1}$ of bee. Letter on x axis indicates significant ifference among the castes for the a given life-history stage. Asterisk indicates significant change from previous life-history stages within the same caste with $* p<0.05, * * p<0.01,{ }^{* * *} p<0.001$. Numbers of replicates per group are provided in Table 1.


Figure 3- Amount of the main phospholipid classes in lipid extract of the three different castes of honey bees (Apis mellifera). Phospholipid classes are A: Phosphatidylcholine (PC); B: phosphatidylethanolamine (PE); C: phosphatidylinositol (PIn) and D: phosphatidylserine (PS). Each caste has six life-history stages characterised. Stages are described as (from left to right): early larva (EL), late larva (LL), pupa (PU), emergent adult (EA), young adult (YA) and old adult (OA) bees. Data are presented as mean $\pm$ s.e.m. and expressed as nmol $\cdot \mathrm{mg}^{-1}$ of bee. Letter on x axis indicates significant difference among the castes for the a given life-history stage. Asterisk indicates significant change from previous life-history stages within the same caste with $* p<0.05, * * p<0.01$, $* * * p<0.001$. Numbers of replicates per group are provided in Table 1.


Figure 4- Contribution of the different phospholipid headgroups on the peroxidation index of membrane phospholipid extract of the three different castes of honey bees (Apis mellifera). Each caste has six life-history stages characterised. Stages are described as (from left to right): early larva (EL), late larva (LL), pupa (PU), emergent adult (EA), young adult (YA) and old adult (OA) bees. Dash line indicates the emergence as adult. Data are presented as mean and expressed as proportion of phospholipids of membrane peroxidation index (PI). Numbers of replicates per group are provided in Table 1.

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

|  | nmol per <br> sample |
| :--- | :--- |
| Molecular phospholipids | 15 |
| LPC 17:0 | 80 |
| PC 19:0_19:0 |  |
| 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, $N L$ neutral loss, $D P$ declustering potential, $E P$ entrance potential, $C E$ collision energy, $C X P$ collision cell exit potential, $P C$ phosphatidylcholine; $P E$ phosphatidylethanolamine; $P S$ 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).


Data are expressed in nmol mg and as mean $\pm$ 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 |  |  | Workers | Queens | Drones |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Early larva | $0.14 \pm 0.03^{\text {a }}$ | $0.14 \pm 0.05^{\text {a }}$ | ND | $\begin{aligned} & \ddot{\ddot{\theta}} \\ & \underset{\vdots}{\dot{\theta}} \\ & \dot{\sim} \end{aligned}$ | Early larva | $0.12 \pm 0.01^{\text {a }}$ | $0.09 \pm 0.03^{\text {ab }}$ | $0.07 \pm 0.01^{\text {b }}$ |
|  | Late larva | $0.001 \pm 0.0001 * * *$ | ND | ND |  | Late larva | $0.05 \pm 0.002^{* * *}$ | ND | $0.07 \pm 0.01$ |
|  | Pupa | $0.0004 \pm 0.0001$ | ND | ND |  | Pupa | $0.04 \pm 0.002$ | ND | $0.06 \pm 0.01$ |
|  | Emergent adult | $0.02 \pm 0.002$ | ND | ND |  | Emergent adult | $0.03 \pm 0.001^{\text {a }}$ | ND | $0.06 \pm 0.001^{\text {b }}$ |
|  | Young adult | $0.005 \pm 0.0004$ | ND | ND |  | Young adult | $0.01 \pm 0.002^{\text {a }}$ | ND | $0.05 \pm 0.003^{\text {b }}$ |
|  | Old adult | $0.03 \pm 0.01$ | ND | ND |  | Old adult | $0.03 \pm 0.003$ | ND | $0.04 \pm 0.002$ |
| $\begin{aligned} & \underset{\dot{0}}{\underline{1}} \\ & \ddot{0} \\ & \dot{0} \\ & \dot{a} \end{aligned}$ | Early larva | $0.15 \pm 0.03$ | $0.13 \pm 0.03$ | $0.20 \pm 0.02$ |  | Early larva | ND | $0.010 \pm 0.003$ | ND |
|  | Late larva | $0.002 \pm 0.0003^{* * *}$ | $0.20 \pm 0.03$ | $0.14 \pm 0.03$ |  | Late larva | ND | $0.003 \pm 0.0002$ | ND |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.32 \pm 0.03^{\text {b }}$ | $0.25 \pm 0.09^{\text {b }}$ |  | Pupa | ND | $0.002 \pm 0.0005^{\text {a }}$ | $0.03 \pm 0.01^{\mathrm{b} * * *}$ |
|  | Emergent adult | $0.05 \pm 0.006^{\text {a }}$ | $0.48 \pm 0.1^{\text {b }}$ | $0.25 \pm 0.02^{\text {c }}$ |  | Emergent adult | $0.004 \pm 0.0003^{\text {a }}$ | $0.004 \pm 0.001^{\text {a }}$ | $0.04 \pm 0.001^{\text {b }}$ |
|  | Young adult | $0.02 \pm 0.0005^{\text {a }}$ | $0.60 \pm 0.1^{\text {b }}$ | $0.04 \pm 0.004^{\text {a****}}$ |  | Young adult | $0.002 \pm 0.0003^{\text {a }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.01 \pm 0.002^{\text {ab }}$ |
|  | Old adult | $0.04 \pm 0.005^{\text {a }}$ | $0.35 \pm 0.04^{\text {b*** }}$ | $0.05 \pm 0.008^{\text {a }}$ |  | Old adult | $0.003 \pm 0.0005$ | $0.01 \pm 0.001$ | $0.01 \pm 0.001$ |
| $\begin{aligned} & \underset{\infty}{\ddot{\infty}} \\ & \underset{\sim}{\ddot{0}} \\ & \underset{a}{n} \end{aligned}$ | Early larva | ND | ND | ND | $\begin{aligned} & \overrightarrow{\dot{\theta}} \\ & \underset{\sim}{\ddot{\theta}} \\ & \underset{\sim}{n} \end{aligned}$ | Early larva | $0.4 \pm 0.04$ | $0.3 \pm 0.08$ | $0.3 \pm 0.1$ |
|  | Late larva | ND | ND | ND |  | Late larva | $0.3 \pm 0.02$ | $0.3 \pm 0.02$ | $0.3 \pm 0.04$ |
|  | Pupa | ND | ND | ND |  | Pupa | $0.2 \pm 0.01$ | $0.2 \pm 0.03$ | $0.1 \pm 0.1$ |
|  | Emergent adult | ND | ND | ND |  | Emergent adult | $0.3 \pm 0.01$ | $0.4 \pm 0.18$ | $0.02 \pm 0.001$ |
|  | Young adult | ND | ND | ND |  | Young adult | $0.2 \pm 0.01^{\text {a }}$ | $0.5 \pm 0.04{ }^{\text {b }}$ | $0.4 \pm 0.01{ }^{\text {ab }}$ |
|  | Old adult | ND | ND | ND |  | Old adult | $0.3 \pm 0.02$ | $0.4 \pm 0.03$ | $0.3 \pm 0.01$ |
|  | Early larva | $0.16 \pm 0.02$ | $0.1 \pm 0.03$ | $0.16 \pm 0.01$ |  | Early larva | $0.3 \pm 0.03$ | $0.3 \pm 0.1$ | $0.2 \pm 0.04$ |
|  | Late larva | $0.001 \pm 0.0002^{\text {a } * * *}$ | $0.2 \pm 0.02^{\text {b }}$ | $0.07 \pm 0.02^{\text {c*** }}$ |  | Late larva | $0.2 \pm 0.01^{\text {a*** }}$ | $0.1 \pm 0.01^{\text {a****}}$ | $0.2 \pm 0.03^{\text {b }}$ |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.3 \pm 0.01^{\mathrm{b} * * *}$ | $0.05 \pm 0.01^{\text {a }}$ |  | Pupa | $0.2 \pm 0.01$ | $0.1 \pm 0.02$ | $0.1 \pm 0.06$ |
|  | Emergent adult | $0.04 \pm 0.004^{\text {a*** }}$ | $0.4 \pm 0.04{ }^{\text {b }}$ | $0.4 \pm 0.01^{\text {b*** }}$ |  | Emergent adult | $0.2 \pm 0.004 *$ | $0.3 \pm 0.15$ | $0.02 \pm 0.001$ |
|  | Young adult | $0.02 \pm 0.001$ | $0.006 \pm 0.001^{* * *}$ | $0.04 \pm 0.003^{* * *}$ |  | Young adult | $0.1 \pm 0.002^{\text {a }}$ | $0.3 \pm 0.03^{\text {b }}$ | $0.3 \pm 0.01^{\mathrm{b} * * *}$ |
|  | Old adult | $0.03 \pm 0.003$ | $0.004 \pm 0.001$ | $0.06 \pm 0.01$ |  | Old adult | $0.2 \pm 0.01^{\text {a****}}$ | $0.2 \pm 0.02^{\text {a } * * *}$ | $0.2 \pm 0.01^{\text {b }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \dddot{\infty} \\ & \underset{\sim}{0} \\ & \dot{\theta} \\ & \ddot{U} \\ & \ddot{Q} \end{aligned}$ | Early larva | $0.04 \pm 0.005$ | ND | $0.03 \pm 0.01$ |  | Early larva | ND | ND | ND |
|  | Late larva | $0.06 \pm 0.005$ | $0.02 \pm 0.002$ | $0.03 \pm 0.01$ |  | Late larva | ND | ND | ND |
|  | Pupa | $0.05 \pm 0.004$ | $0.01 \pm 0.001$ | $0.06 \pm 0.01$ |  | Pupa | ND | ND | $0.01 \pm 0.001^{\text {b }}$ |
|  | Emergent adult | $0.06 \pm 0.003$ | $0.02 \pm 0.004$ | $0.04 \pm 0.004$ |  | Emergent adult | ND | $0.002 \pm 0.001^{\text {a }}$ | $0.004 \pm 0.0004^{\text {ab****}}$ |
|  | Young adult | $0.2 \pm 0.02^{* * *}$ | $0.1 \pm 0.01$ * | $0.09 \pm 0.02$ |  | Young adult | ND | $0.01 \pm 0.001^{\text {a*** }}$ | $0.01 \pm 0.001^{\text {b }}$ |
|  | Old adult | $0.4 \pm 0.033^{\text {a****}}$ | $0.1 \pm 0.004^{\text {b }}$ | ND |  | Old adult | ND | $0.02 \pm 0.002^{\text {a*** }}$ | $0.01 \pm 0.001^{\text {b }}$ |
| $\begin{aligned} & \underset{\infty}{0} \\ & \underset{\sim}{\prime} \\ & \dot{\theta} \\ & \dot{U} \\ & \dot{Q} \end{aligned}$ | Early larva | ND | ND | ND |  | Early larva | ND | $0.02 \pm 0.001$ | $0.03 \pm 0.002$ |
|  | Late larva | ND | $0.01 \pm 0.001$ | ND |  | Late larva | ND | $0.02 \pm 0.002$ | $0.02 \pm 0.002$ |
|  | Pupa | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.02 \pm 0.004$ |  | Pupa | ND | $0.02 \pm 0.002$ | $0.03 \pm 0.005$ |
|  | Emergent adult | $0.04 \pm 0.001$ | $0.01 \pm 0.003$ | $0.02 \pm 0.003$ |  | Emergent adult | $0.1 \pm 0.003$ | $0.02 \pm 0.005$ | $0.05 \pm 0.005$ |
|  | Young adult | $0.08 \pm 0.005^{\text {a }}$ | $0.05 \pm 0.004^{\text {a }}$ | $0.4 \pm 0.04^{\text {a } * * *}$ |  | Young adult | $0.2 \pm 0.01 *$ | $0.10 \pm 0.01^{* * *}$ | $0.09 \pm 0.01$ |
|  | Old adult | $0.35 \pm 0.04^{\text {a } * * *}$ | $0.06 \pm 0.005^{\text {b }}$ | $0.3 \pm 0.02^{\text {a }}$ |  | Old adult | $0.5 \pm 0.04^{\text {a****}}$ | $0.12 \pm 0.01^{\text {b }}$ | $0.08 \pm 0.005^{\text {b }}$ |
|  | Early larva | $0.05 \pm 0.01$ | $0.06 \pm 0.02$ | $0.05 \pm 0.002$ | $\begin{gathered} \underset{\sim}{\dot{\infty}} \\ \underset{\sim}{\sim} \\ \underset{\sim}{\dot{\infty}} \\ \hline \end{gathered}$ | Early larva | $0.20 \pm 0.02^{\text {a }}$ | $0.03 \pm 0.001^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |
|  | Late larva | $0.14 \pm 0.01$ | $0.02 \pm 0.003$ | $0.05 \pm 0.01$ |  | Late larva | $0.07 \pm 0.01^{\text {a }}$ | $0.003 \pm 0.001^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |
|  | Pupa | $0.10 \pm 0.004$ | $0.03 \pm 0.004$ | $0.16 \pm 0.03$ |  | Pupa | $0.07 \pm 0.003^{\text {a }}$ | $0.01 \pm 0.001^{\text {b }}$ | $0.03 \pm 0.004^{\text {b }}$ |
|  | Emergent adult | $0.12 \pm 0.01^{\text {a }}$ | $0.05 \pm 0.01^{\text {a }}$ | $0.40 \pm 0.05^{\text {b*** }}$ |  | Emergent adult | $0.04 \pm 0.004^{\text {a }}$ | $0.01 \pm 0.003^{\text {a }}$ | $0.07 \pm 0.01^{\text {b }}$ |
|  | Young adult | $0.41 \pm 0.02^{\text {a **** }}$ | $0.20 \pm 0.01^{\text {b* }}$ | $0.03 \pm 0.003^{\text {c*** }}$ |  | Young adult | $0.16 \pm 0.01^{\text {a*** }}$ | $0.07 \pm 0.01^{\text {b } * * *}$ | $0.10 \pm 0.01^{\text {b }}$ |
|  | Old adult | $0.94 \pm 0.06^{\text {a *** }}$ | $0.22 \pm 0.02^{\text {b }}$ | $0.04 \pm 0.003^{\text {c }}$ |  | Old adult | $0.25 \pm 0.01^{\text {a*** }}$ | $0.08 \pm 0.01 \mathrm{~b}$ | $0.10 \pm 0.01^{\text {b }}$ |
| $m$0$\sim$-1000 | Early larva | $0.11 \pm 0.01$ | $0.07 \pm 0.002$ | $0.16 \pm 0.01$ | $\begin{gathered} \underset{\infty}{\infty} \\ \underset{\sim}{1} \\ \underset{\sim}{\infty} \\ \underset{\sim}{u} \end{gathered}$ | Early larva | ND | $0.04 \pm 0.004$ | $0.06 \pm 0.002$ |
|  | Late larva | $0.21 \pm 0.02$ | $0.07 \pm 0.01$ | $0.13 \pm 0.01$ |  | Late larva | ND | $0.02 \pm 0.002^{\text {a }}$ | $0.05 \pm 0.002^{\text {b }}$ |
|  | Pupa | $0.14 \pm 0.01$ | $0.04 \pm 0.01$ | $0.23 \pm 0.1$ |  | Pupa | ND | $0.01 \pm 0.002$ | $0.04 \pm 0.01$ |
|  | Emergent adult | $0.19 \pm 0.01$ | $0.07 \pm 0.01$ | $0.14 \pm 0.01$ |  | Emergent adult | $0.07 \pm 0.003^{\text {a }}$ | $0.01 \pm 0.004^{\text {b }}$ | $0.04 \pm 0.003^{\text {b }}$ |
|  | Young adult | $0.74 \pm 0.04^{\text {a **** }}$ | $0.42 \pm 0.04^{\mathrm{b} * * *}$ | $0.33 \pm 0.04{ }^{\text {b }}$ |  | Young adult | $0.26 \pm 0.01^{\text {a****}}$ | $0.14 \pm 0.02^{\text {b****}}$ | $0.12 \pm 0.01^{\text {b }}$ |
|  | Old adult | $1.58 \pm 0.12^{\text {a } * * *}$ | $0.55 \pm 0.06^{\text {b }}$ | $0.25 \pm 0.02^{\text {c }}$ |  | Old adult | $0.45 \pm 0.03^{\text {a*** }}$ | $0.19 \pm 0.03^{\text {b }}$ | $0.11 \pm 0.01^{\text {c }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| - | Early larva | $0.04 \pm 0.1$ | ND | $0.03 \pm 0.002$ | $\begin{aligned} & \tilde{0} \\ & \dot{\theta} \\ & \dot{\theta} \\ & \dot{\theta} \\ & \dot{\sim} \end{aligned}$ | Early larva | ND | ND | ND |
|  | Late larva | $0.0005 \pm 0.0001$ | $0.06 \pm 0.01$ | $0.02 \pm 0.004$ |  | Late larva | ND | ND | ND |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.05 \pm 0.004^{\text {a }}$ | $0.12 \pm 0.03^{\text {b } * * *}$ |  | Pupa | ND | ND | ND |
|  | Emergent adult | $0.02 \pm 0.002^{\text {a }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.10 \pm 0.01^{\text {b }}$ |  | Emergent adult | ND | ND | ND |
|  | Young adult | $0.02 \pm 0.002^{\text {a }}$ | $0.2 \pm 0.03^{\text {b*** }}$ | $0.02 \pm 0.003^{\text {a*** }}$ |  | Young adult | ND | ND | ND |
|  | Old adult | $0.03 \pm 0.005^{\text {a }}$ | $0.11 \pm 0.02^{\text {b* }}$ | $0.02 \pm 0.002^{\text {a }}$ |  | Old adult | ND | ND | ND |
|  | Early larva | $0.06 \pm 0.01$ | ND | $0.1 \pm 0.01$ | $n$$\ddot{\infty}$00$\dot{\infty}$$\vdots$$\sim$ | Early larva | ND | ND | ND |
|  | Late larva | $0.001 \pm 0.0002$ | $0.15 \pm 0.02$ | $0.06 \pm 0.01$ |  | Late larva | $0.01 \pm 0.002$ | $0.004 \pm 0.000$ | ND |
|  | Pupa | $0.001 \pm 0.0002$ | $0.11 \pm 0.01$ | $0.40 \pm 0.1^{* * *}$ |  | Pupa | $0.02 \pm 0.002$ | $0.007 \pm 0.001$ | $0.01 \pm 0.004$ |
|  | Emergent adult | $0.20 \pm 0.02^{\text {a *** }}$ | $0.10 \pm 0.01^{\text {a }}$ | $0.60 \pm 0.04^{\text {b }}$ |  | Emergent adult | $0.03 \pm 0.001$ | $0.005 \pm 0.002$ | $0.01 \pm 0.000$ |
|  | Young adult | $0.09 \pm 0.01^{\text {a }}$ | $0.96 \pm 0.2^{\text {b*** }}$ | $0.11 \pm 0.01^{\text {a****}}$ |  | Young adult | $0.04 \pm 0.003$ | $0.03 \pm 0.003$ | $0.04 \pm 0.003$ |
|  | Old adult | $0.30 \pm 0.04^{\text {a *** }}$ | $0.65 \pm 0.1^{\text {b } * * *}$ | $0.10 \pm 0.01^{\text {c }}$ |  | Old adult | $0.11 \pm 0.01^{\text {a*** }}$ | $0.04 \pm 0.003^{\text {b }}$ | $0.04 \pm 0.002^{\text {b }}$ |
|  | Early larva | ND | ND | ND | $\begin{gathered} \underset{\sim}{\dot{\infty}} \\ \underset{\sim}{\ddot{\infty}} \\ \dot{\sim} \\ \dot{\sim} \end{gathered}$ | Early larva | ND | ND | ND |
|  | Late larva | ND | $0.01 \pm 0.001$ | ND |  | Late larva | ND | ND | ND |
|  | Pupa | ND | $0.03 \pm 0.003$ | $0.05 \pm 0.02$ |  | Pupa | ND | $0.001 \pm 0.000$ | $0.15 \pm 0.04 * * *$ |
|  | Emergent adult | ND | $0.003 \pm 0.003$ | $0.09 \pm 0.01$ |  | Emergent adult | $0.01 \pm 0.001^{\text {a }}$ | $0.004 \pm 0.001 \mathrm{a}$ | $0.17 \pm 0.005^{\text {b }}$ |
|  | Young adult | ND | $0.05 \pm 0.01$ | $0.03 \pm 0.003$ |  | Young adult | $0.01 \pm 0.002$ | $0.01 \pm 0.001$ | $0.01 \pm 0.001^{* * *}$ |
|  | Old adult | ND | $0.04 \pm 0.01$ | $0.03 \pm 0.003$ |  | Old adult | $0.02 \pm 0.001$ | $0.01 \pm 0.000$ | $0.01 \pm 0.001$ |
|  | Early larva | ND | ND | $0.03 \pm 0.003$ |  | Early larva | ND | ND | ND |
|  | Late larva | $0.004 \pm 0.001$ | $0.05 \pm 0.01$ | $0.02 \pm 0.003$ |  | Late larva | $0.004 \pm 0.001$ | $0.003 \pm 0.001$ | ND |
|  | Pupa | $0.01 \pm 0.001^{\text {a }}$ | $0.05 \pm 0.005^{\text {a }}$ | $0.13 \pm 0.03^{\text {b } * * *}$ |  | Pupa | $0.01 \pm 0.001$ | $0.004 \pm 0.001$ | $0.01 \pm 0.003$ |
|  | Emergent adult | $0.02 \pm 0.0004^{\text {a }}$ | $0.04 \pm 0.003^{\text {a }}$ | $0.16 \pm 0.01^{\text {b }}$ |  | Emergent adult | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.01 \pm 0.000$ |
|  | Young adult | $0.02 \pm 0.001^{\text {a }}$ | $0.20 \pm 0.03^{\text {b*** }}$ | $0.03 \pm 0.003^{\text {a*** }}$ |  | Young adult | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.02 \pm 0.001$ |
|  | Old adult | $0.04 \pm 0.002^{\text {a }}$ | $0.16 \pm 0.03^{\text {b }}$ | $0.04 \pm 0.005^{\text {a }}$ |  | Old adult | $0.04 \pm 0.002^{\text {a*** }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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.

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

|  | nmol per <br> sample |
| :--- | :--- |
| Molecular phospholipids | 15 |
| LPC 17:0 | 80 |
| PC 19:0_19:0 |  |
| 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, $N L$ neutral loss, $D P$ declustering potential, $E P$ entrance potential, $C E$ collision energy, $C X P$ collision cell exit potential, $P C$ phosphatidylcholine; $P E$ phosphatidylethanolamine; $P S$ 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).


Data are expressed in nmol mg and as mean $\pm$ 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 |  |  | Workers | Queens | Drones |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Early larva | $0.14 \pm 0.03^{\text {a }}$ | $0.14 \pm 0.05^{\text {a }}$ | ND | $\begin{aligned} & \ddot{\ddot{\theta}} \\ & \underset{\vdots}{\dot{\theta}} \\ & \dot{\sim} \end{aligned}$ | Early larva | $0.12 \pm 0.01^{\text {a }}$ | $0.09 \pm 0.03^{\text {ab }}$ | $0.07 \pm 0.01^{\text {b }}$ |
|  | Late larva | $0.001 \pm 0.0001 * * *$ | ND | ND |  | Late larva | $0.05 \pm 0.002^{* * *}$ | ND | $0.07 \pm 0.01$ |
|  | Pupa | $0.0004 \pm 0.0001$ | ND | ND |  | Pupa | $0.04 \pm 0.002$ | ND | $0.06 \pm 0.01$ |
|  | Emergent adult | $0.02 \pm 0.002$ | ND | ND |  | Emergent adult | $0.03 \pm 0.001^{\text {a }}$ | ND | $0.06 \pm 0.001^{\text {b }}$ |
|  | Young adult | $0.005 \pm 0.0004$ | ND | ND |  | Young adult | $0.01 \pm 0.002^{\text {a }}$ | ND | $0.05 \pm 0.003^{\text {b }}$ |
|  | Old adult | $0.03 \pm 0.01$ | ND | ND |  | Old adult | $0.03 \pm 0.003$ | ND | $0.04 \pm 0.002$ |
| $\begin{aligned} & \underset{\dot{0}}{\underline{1}} \\ & \ddot{0} \\ & \dot{0} \\ & \dot{a} \end{aligned}$ | Early larva | $0.15 \pm 0.03$ | $0.13 \pm 0.03$ | $0.20 \pm 0.02$ |  | Early larva | ND | $0.010 \pm 0.003$ | ND |
|  | Late larva | $0.002 \pm 0.0003^{* * *}$ | $0.20 \pm 0.03$ | $0.14 \pm 0.03$ |  | Late larva | ND | $0.003 \pm 0.0002$ | ND |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.32 \pm 0.03^{\text {b }}$ | $0.25 \pm 0.09^{\text {b }}$ |  | Pupa | ND | $0.002 \pm 0.0005^{\text {a }}$ | $0.03 \pm 0.01^{\mathrm{b} * * *}$ |
|  | Emergent adult | $0.05 \pm 0.006^{\text {a }}$ | $0.48 \pm 0.1^{\text {b }}$ | $0.25 \pm 0.02^{\text {c }}$ |  | Emergent adult | $0.004 \pm 0.0003^{\text {a }}$ | $0.004 \pm 0.001^{\text {a }}$ | $0.04 \pm 0.001^{\text {b }}$ |
|  | Young adult | $0.02 \pm 0.0005^{\text {a }}$ | $0.60 \pm 0.1^{\text {b }}$ | $0.04 \pm 0.004^{\text {a****}}$ |  | Young adult | $0.002 \pm 0.0003^{\text {a }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.01 \pm 0.002^{\text {ab }}$ |
|  | Old adult | $0.04 \pm 0.005^{\text {a }}$ | $0.35 \pm 0.04^{\text {b*** }}$ | $0.05 \pm 0.008^{\text {a }}$ |  | Old adult | $0.003 \pm 0.0005$ | $0.01 \pm 0.001$ | $0.01 \pm 0.001$ |
| $\begin{aligned} & \underset{\infty}{\ddot{\infty}} \\ & \underset{\sim}{\ddot{0}} \\ & \underset{a}{n} \end{aligned}$ | Early larva | ND | ND | ND | $\begin{aligned} & \overrightarrow{\dot{\theta}} \\ & \underset{\sim}{\ddot{\theta}} \\ & \underset{\sim}{n} \end{aligned}$ | Early larva | $0.4 \pm 0.04$ | $0.3 \pm 0.08$ | $0.3 \pm 0.1$ |
|  | Late larva | ND | ND | ND |  | Late larva | $0.3 \pm 0.02$ | $0.3 \pm 0.02$ | $0.3 \pm 0.04$ |
|  | Pupa | ND | ND | ND |  | Pupa | $0.2 \pm 0.01$ | $0.2 \pm 0.03$ | $0.1 \pm 0.1$ |
|  | Emergent adult | ND | ND | ND |  | Emergent adult | $0.3 \pm 0.01$ | $0.4 \pm 0.18$ | $0.02 \pm 0.001$ |
|  | Young adult | ND | ND | ND |  | Young adult | $0.2 \pm 0.01^{\text {a }}$ | $0.5 \pm 0.04{ }^{\text {b }}$ | $0.4 \pm 0.01{ }^{\text {ab }}$ |
|  | Old adult | ND | ND | ND |  | Old adult | $0.3 \pm 0.02$ | $0.4 \pm 0.03$ | $0.3 \pm 0.01$ |
|  | Early larva | $0.16 \pm 0.02$ | $0.1 \pm 0.03$ | $0.16 \pm 0.01$ |  | Early larva | $0.3 \pm 0.03$ | $0.3 \pm 0.1$ | $0.2 \pm 0.04$ |
|  | Late larva | $0.001 \pm 0.0002^{\text {a } * * *}$ | $0.2 \pm 0.02^{\text {b }}$ | $0.07 \pm 0.02^{\text {c*** }}$ |  | Late larva | $0.2 \pm 0.01^{\text {a*** }}$ | $0.1 \pm 0.01^{\text {a****}}$ | $0.2 \pm 0.03^{\text {b }}$ |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.3 \pm 0.01^{\mathrm{b} * * *}$ | $0.05 \pm 0.01^{\text {a }}$ |  | Pupa | $0.2 \pm 0.01$ | $0.1 \pm 0.02$ | $0.1 \pm 0.06$ |
|  | Emergent adult | $0.04 \pm 0.004^{\text {a*** }}$ | $0.4 \pm 0.04{ }^{\text {b }}$ | $0.4 \pm 0.01^{\text {b*** }}$ |  | Emergent adult | $0.2 \pm 0.004 *$ | $0.3 \pm 0.15$ | $0.02 \pm 0.001$ |
|  | Young adult | $0.02 \pm 0.001$ | $0.006 \pm 0.001^{* * *}$ | $0.04 \pm 0.003^{* * *}$ |  | Young adult | $0.1 \pm 0.002^{\text {a }}$ | $0.3 \pm 0.03^{\text {b }}$ | $0.3 \pm 0.01^{\mathrm{b} * * *}$ |
|  | Old adult | $0.03 \pm 0.003$ | $0.004 \pm 0.001$ | $0.06 \pm 0.01$ |  | Old adult | $0.2 \pm 0.01^{\text {a****}}$ | $0.2 \pm 0.02^{\text {a } * * *}$ | $0.2 \pm 0.01^{\text {b }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \dddot{\infty} \\ & \underset{\sim}{0} \\ & \dot{\theta} \\ & \ddot{U} \\ & \ddot{Q} \end{aligned}$ | Early larva | $0.04 \pm 0.005$ | ND | $0.03 \pm 0.01$ |  | Early larva | ND | ND | ND |
|  | Late larva | $0.06 \pm 0.005$ | $0.02 \pm 0.002$ | $0.03 \pm 0.01$ |  | Late larva | ND | ND | ND |
|  | Pupa | $0.05 \pm 0.004$ | $0.01 \pm 0.001$ | $0.06 \pm 0.01$ |  | Pupa | ND | ND | $0.01 \pm 0.001^{\text {b }}$ |
|  | Emergent adult | $0.06 \pm 0.003$ | $0.02 \pm 0.004$ | $0.04 \pm 0.004$ |  | Emergent adult | ND | $0.002 \pm 0.001^{\text {a }}$ | $0.004 \pm 0.0004^{\text {ab****}}$ |
|  | Young adult | $0.2 \pm 0.02^{* * *}$ | $0.1 \pm 0.01$ * | $0.09 \pm 0.02$ |  | Young adult | ND | $0.01 \pm 0.001^{\text {a*** }}$ | $0.01 \pm 0.001^{\text {b }}$ |
|  | Old adult | $0.4 \pm 0.033^{\text {a****}}$ | $0.1 \pm 0.004^{\text {b }}$ | ND |  | Old adult | ND | $0.02 \pm 0.002^{\text {a*** }}$ | $0.01 \pm 0.001^{\text {b }}$ |
| $\begin{aligned} & \underset{\infty}{0} \\ & \underset{\sim}{\prime} \\ & \dot{\theta} \\ & \dot{U} \\ & \dot{Q} \end{aligned}$ | Early larva | ND | ND | ND |  | Early larva | ND | $0.02 \pm 0.001$ | $0.03 \pm 0.002$ |
|  | Late larva | ND | $0.01 \pm 0.001$ | ND |  | Late larva | ND | $0.02 \pm 0.002$ | $0.02 \pm 0.002$ |
|  | Pupa | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.02 \pm 0.004$ |  | Pupa | ND | $0.02 \pm 0.002$ | $0.03 \pm 0.005$ |
|  | Emergent adult | $0.04 \pm 0.001$ | $0.01 \pm 0.003$ | $0.02 \pm 0.003$ |  | Emergent adult | $0.1 \pm 0.003$ | $0.02 \pm 0.005$ | $0.05 \pm 0.005$ |
|  | Young adult | $0.08 \pm 0.005^{\text {a }}$ | $0.05 \pm 0.004^{\text {a }}$ | $0.4 \pm 0.04^{\text {a } * * *}$ |  | Young adult | $0.2 \pm 0.01 *$ | $0.10 \pm 0.01^{* * *}$ | $0.09 \pm 0.01$ |
|  | Old adult | $0.35 \pm 0.04^{\text {a } * * *}$ | $0.06 \pm 0.005^{\text {b }}$ | $0.3 \pm 0.02^{\text {a }}$ |  | Old adult | $0.5 \pm 0.04^{\text {a****}}$ | $0.12 \pm 0.01^{\text {b }}$ | $0.08 \pm 0.005^{\text {b }}$ |
|  | Early larva | $0.05 \pm 0.01$ | $0.06 \pm 0.02$ | $0.05 \pm 0.002$ | $\begin{gathered} \underset{\sim}{\dot{\infty}} \\ \underset{\sim}{\sim} \\ \underset{\sim}{\dot{\infty}} \\ \hline \end{gathered}$ | Early larva | $0.20 \pm 0.02^{\text {a }}$ | $0.03 \pm 0.001^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |
|  | Late larva | $0.14 \pm 0.01$ | $0.02 \pm 0.003$ | $0.05 \pm 0.01$ |  | Late larva | $0.07 \pm 0.01^{\text {a }}$ | $0.003 \pm 0.001^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |
|  | Pupa | $0.10 \pm 0.004$ | $0.03 \pm 0.004$ | $0.16 \pm 0.03$ |  | Pupa | $0.07 \pm 0.003^{\text {a }}$ | $0.01 \pm 0.001^{\text {b }}$ | $0.03 \pm 0.004^{\text {b }}$ |
|  | Emergent adult | $0.12 \pm 0.01^{\text {a }}$ | $0.05 \pm 0.01^{\text {a }}$ | $0.40 \pm 0.05^{\text {b*** }}$ |  | Emergent adult | $0.04 \pm 0.004^{\text {a }}$ | $0.01 \pm 0.003^{\text {a }}$ | $0.07 \pm 0.01^{\text {b }}$ |
|  | Young adult | $0.41 \pm 0.02^{\text {a **** }}$ | $0.20 \pm 0.01^{\text {b* }}$ | $0.03 \pm 0.003^{\text {c*** }}$ |  | Young adult | $0.16 \pm 0.01^{\text {a*** }}$ | $0.07 \pm 0.01^{\text {b } * * *}$ | $0.10 \pm 0.01^{\text {b }}$ |
|  | Old adult | $0.94 \pm 0.06^{\text {a *** }}$ | $0.22 \pm 0.02^{\text {b }}$ | $0.04 \pm 0.003^{\text {c }}$ |  | Old adult | $0.25 \pm 0.01^{\text {a*** }}$ | $0.08 \pm 0.01 \mathrm{~b}$ | $0.10 \pm 0.01^{\text {b }}$ |
| $m$0$\sim$-1000 | Early larva | $0.11 \pm 0.01$ | $0.07 \pm 0.002$ | $0.16 \pm 0.01$ | $\begin{gathered} \underset{\infty}{\infty} \\ \underset{\sim}{1} \\ \underset{\sim}{\infty} \\ \underset{\sim}{u} \end{gathered}$ | Early larva | ND | $0.04 \pm 0.004$ | $0.06 \pm 0.002$ |
|  | Late larva | $0.21 \pm 0.02$ | $0.07 \pm 0.01$ | $0.13 \pm 0.01$ |  | Late larva | ND | $0.02 \pm 0.002^{\text {a }}$ | $0.05 \pm 0.002^{\text {b }}$ |
|  | Pupa | $0.14 \pm 0.01$ | $0.04 \pm 0.01$ | $0.23 \pm 0.1$ |  | Pupa | ND | $0.01 \pm 0.002$ | $0.04 \pm 0.01$ |
|  | Emergent adult | $0.19 \pm 0.01$ | $0.07 \pm 0.01$ | $0.14 \pm 0.01$ |  | Emergent adult | $0.07 \pm 0.003^{\text {a }}$ | $0.01 \pm 0.004^{\text {b }}$ | $0.04 \pm 0.003^{\text {b }}$ |
|  | Young adult | $0.74 \pm 0.04^{\text {a **** }}$ | $0.42 \pm 0.04^{\mathrm{b} * * *}$ | $0.33 \pm 0.04{ }^{\text {b }}$ |  | Young adult | $0.26 \pm 0.01^{\text {a****}}$ | $0.14 \pm 0.02^{\text {b****}}$ | $0.12 \pm 0.01^{\text {b }}$ |
|  | Old adult | $1.58 \pm 0.12^{\text {a } * * *}$ | $0.55 \pm 0.06^{\text {b }}$ | $0.25 \pm 0.02^{\text {c }}$ |  | Old adult | $0.45 \pm 0.03^{\text {a*** }}$ | $0.19 \pm 0.03^{\text {b }}$ | $0.11 \pm 0.01^{\text {c }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| - | Early larva | $0.04 \pm 0.1$ | ND | $0.03 \pm 0.002$ | $\begin{aligned} & \tilde{0} \\ & \dot{\theta} \\ & \dot{\theta} \\ & \dot{\theta} \\ & \dot{\sim} \end{aligned}$ | Early larva | ND | ND | ND |
|  | Late larva | $0.0005 \pm 0.0001$ | $0.06 \pm 0.01$ | $0.02 \pm 0.004$ |  | Late larva | ND | ND | ND |
|  | Pupa | $0.0003 \pm 0.0001^{\text {a }}$ | $0.05 \pm 0.004^{\text {a }}$ | $0.12 \pm 0.03^{\text {b } * * *}$ |  | Pupa | ND | ND | ND |
|  | Emergent adult | $0.02 \pm 0.002^{\text {a }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.10 \pm 0.01^{\text {b }}$ |  | Emergent adult | ND | ND | ND |
|  | Young adult | $0.02 \pm 0.002^{\text {a }}$ | $0.2 \pm 0.03^{\text {b*** }}$ | $0.02 \pm 0.003^{\text {a*** }}$ |  | Young adult | ND | ND | ND |
|  | Old adult | $0.03 \pm 0.005^{\text {a }}$ | $0.11 \pm 0.02^{\text {b* }}$ | $0.02 \pm 0.002^{\text {a }}$ |  | Old adult | ND | ND | ND |
|  | Early larva | $0.06 \pm 0.01$ | ND | $0.1 \pm 0.01$ | $n$$\ddot{\infty}$00$\dot{\infty}$$\vdots$$\sim$ | Early larva | ND | ND | ND |
|  | Late larva | $0.001 \pm 0.0002$ | $0.15 \pm 0.02$ | $0.06 \pm 0.01$ |  | Late larva | $0.01 \pm 0.002$ | $0.004 \pm 0.000$ | ND |
|  | Pupa | $0.001 \pm 0.0002$ | $0.11 \pm 0.01$ | $0.40 \pm 0.1^{* * *}$ |  | Pupa | $0.02 \pm 0.002$ | $0.007 \pm 0.001$ | $0.01 \pm 0.004$ |
|  | Emergent adult | $0.20 \pm 0.02^{\text {a *** }}$ | $0.10 \pm 0.01^{\text {a }}$ | $0.60 \pm 0.04^{\text {b }}$ |  | Emergent adult | $0.03 \pm 0.001$ | $0.005 \pm 0.002$ | $0.01 \pm 0.000$ |
|  | Young adult | $0.09 \pm 0.01^{\text {a }}$ | $0.96 \pm 0.2^{\text {b*** }}$ | $0.11 \pm 0.01^{\text {a****}}$ |  | Young adult | $0.04 \pm 0.003$ | $0.03 \pm 0.003$ | $0.04 \pm 0.003$ |
|  | Old adult | $0.30 \pm 0.04^{\text {a *** }}$ | $0.65 \pm 0.1^{\text {b } * * *}$ | $0.10 \pm 0.01^{\text {c }}$ |  | Old adult | $0.11 \pm 0.01^{\text {a*** }}$ | $0.04 \pm 0.003^{\text {b }}$ | $0.04 \pm 0.002^{\text {b }}$ |
|  | Early larva | ND | ND | ND | $\begin{gathered} \underset{\sim}{\dot{\infty}} \\ \underset{\sim}{\ddot{\infty}} \\ \dot{\sim} \\ \dot{\sim} \end{gathered}$ | Early larva | ND | ND | ND |
|  | Late larva | ND | $0.01 \pm 0.001$ | ND |  | Late larva | ND | ND | ND |
|  | Pupa | ND | $0.03 \pm 0.003$ | $0.05 \pm 0.02$ |  | Pupa | ND | $0.001 \pm 0.000$ | $0.15 \pm 0.04 * * *$ |
|  | Emergent adult | ND | $0.003 \pm 0.003$ | $0.09 \pm 0.01$ |  | Emergent adult | $0.01 \pm 0.001^{\text {a }}$ | $0.004 \pm 0.001 \mathrm{a}$ | $0.17 \pm 0.005^{\text {b }}$ |
|  | Young adult | ND | $0.05 \pm 0.01$ | $0.03 \pm 0.003$ |  | Young adult | $0.01 \pm 0.002$ | $0.01 \pm 0.001$ | $0.01 \pm 0.001^{* * *}$ |
|  | Old adult | ND | $0.04 \pm 0.01$ | $0.03 \pm 0.003$ |  | Old adult | $0.02 \pm 0.001$ | $0.01 \pm 0.000$ | $0.01 \pm 0.001$ |
|  | Early larva | ND | ND | $0.03 \pm 0.003$ |  | Early larva | ND | ND | ND |
|  | Late larva | $0.004 \pm 0.001$ | $0.05 \pm 0.01$ | $0.02 \pm 0.003$ |  | Late larva | $0.004 \pm 0.001$ | $0.003 \pm 0.001$ | ND |
|  | Pupa | $0.01 \pm 0.001^{\text {a }}$ | $0.05 \pm 0.005^{\text {a }}$ | $0.13 \pm 0.03^{\text {b } * * *}$ |  | Pupa | $0.01 \pm 0.001$ | $0.004 \pm 0.001$ | $0.01 \pm 0.003$ |
|  | Emergent adult | $0.02 \pm 0.0004^{\text {a }}$ | $0.04 \pm 0.003^{\text {a }}$ | $0.16 \pm 0.01^{\text {b }}$ |  | Emergent adult | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.01 \pm 0.000$ |
|  | Young adult | $0.02 \pm 0.001^{\text {a }}$ | $0.20 \pm 0.03^{\text {b*** }}$ | $0.03 \pm 0.003^{\text {a*** }}$ |  | Young adult | $0.02 \pm 0.001$ | $0.01 \pm 0.001$ | $0.02 \pm 0.001$ |
|  | Old adult | $0.04 \pm 0.002^{\text {a }}$ | $0.16 \pm 0.03^{\text {b }}$ | $0.04 \pm 0.005^{\text {a }}$ |  | Old adult | $0.04 \pm 0.002^{\text {a*** }}$ | $0.02 \pm 0.003^{\text {b }}$ | $0.02 \pm 0.001^{\text {b }}$ |

Data are expressed in nmol mg and as mean $\pm$ 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.

