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RESEARCH ARTICLE

# Juvenile hormone affects age polyethism, ovarian status and cuticular hydrocarbon profile in workers of the wasp *Polybia occidentalis*

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## ABSTRACT

Division of labor is one of the most striking features in the evolution of eusociality. Juvenile hormone (JH) mediates reproductive status and aggression among nestmates in primitively eusocial Hymenoptera (species without morphologically distinct castes). In highly social species it has apparently lost its gonadotropic role and primarily regulates the division of labor in the worker caste. *Polybia occidentalis*, a Neotropical swarm-founding wasp, is an ideal model to understand how JH levels mirror social context and reproductive opportunities because of the absence of a clear morphological caste dimorphism. In this study, we tested the hypothesis that JH influences division of labor, ovary activation and cuticular hydrocarbon profiles of workers. Our observations confirmed that a JH analog (methoprene) and an inhibitor of JH biosynthesis (precocene) affected the cuticular chemical profile associated with age polyethism. Also, methoprene and precocene-I treatment of females influenced ovarian activation differently (individuals treated with methoprene expressed more activated ovaries while precocene treatment did not have significant effect). These results suggest that different hormonal levels induce a differential expression of cuticular chemicals associated with workers' age polyethism, which may be essential for keeping the social cohesion among workers throughout their lives in the colony. Furthermore, JH is likely to play a gonadotropic role in *P. occidentalis*. JH has apparently undergone certain modifications in social Hymenoptera, presenting multifaceted functions in different species.

**KEY WORDS: Epiponini wasps, Hormonal treatment, Methoprene, Precocene**

## INTRODUCTION

Division of labor is a key trait in social insects that has favored their ecological success in almost all terrestrial environments (Wilson, 1971). In several social wasps, the division of labor occurs within the framework of age polyethism, i.e. workers perform specific tasks sequentially during their adult life cycles. Typically, young individuals perform tasks within the nest, and as they grow older, they begin to work outside the nest (Jeanne et al., 1988; Jeanne, 1991; O'Donnell, 2001). In terms of colony fitness, a strict link between age and task is likely not desirable; instead, workers should be able to transition between different tasks with a certain degree of flexibility depending on colony needs, as has been observed in several wasp species (West-Eberhard, 1978; Jeanne, 1991; Nascimento et al., 2005).

Age polyethism is mechanistically driven by factors such as genetics, hormones and the social environment, which may interact and predispose an individual to exhibit a particular set of behavioral phenotypes (Gordon, 1996; Hartfelder and Engels, 1998; Jandt et al., 2014; Gordon, 2016; Mateus et al., 2019). Juvenile hormone (JH) is an insect-specific sesquiterpenoid lipid hormone synthesized by the corpora allata, a pair of glands localized in the retrocerebral complex (Nijhout, 1994). Together with ecdysteroid hormones, it regulates larval growth and metamorphosis as well as reproductive physiology and behaviors and the production of pheromones (Wyatt and Davey, 1996; Hartfelder, 2000; Oliveira et al., 2017; reviewed in Tibbetts et al., 2020). Previous studies have also shown that JH plays an important role in the division of labor, sexual maturation and behavioral maturation in social Hymenoptera (Rutz et al., 1976; Robinson and Vargo, 1997; Giray et al., 2005; Tibbetts et al., 2013; Southon et al., 2020).

In social wasps, JH has been shown to influence transitions between worker tasks by accelerating the behavioral ontogeny in experimentally treated individuals (O'Donnell and Jeanne, 1993; Chang et al., 2015; Giray et al., 2005; Shorter and Tibbetts, 2009; Tibbetts et al., 2013). As JH also causes an increase in the oocyte length of workers by acting as an ovarian activator (Kelstrup et al., 2014b; Oi et al., 2021), both reproductive and behavioral traits are controlled by the same endocrine pathway (O'Donnell and Jeanne, 1993; Kelstrup et al., 2014b). Furthermore, JH also appears to be involved in regulating pheromone production, such as the cuticular hydrocarbon (CHC) profiles of queens and workers (Robinson and Vargo, 1997; Oliveira et al., 2017). CHCs provide nestmates with important information on an individual's caste, reproductive status and functional role in the colony (Singer and Espelie, 1992; Dani et al., 2001; Dapporto et al., 2004; Izzo et al., 2010; Ferreira-Caliman et al., 2010; Oi et al., 2015a; Kather and Martin, 2015; Valadares and Nascimento, 2016; Santos et al., 2018; reviewed in Antonialli-Junior et al., 2021). In primitively eusocial wasps, the CHC profiles of workers are linked to both JH titer and the individual's behavioral role (Sledge et al., 2004; Tibbetts and Izzo, 2009; Izzo et al., 2010). However, in eusocial epiponine and vespine wasps, higher levels of JH are tightly associated with the expression of queen-like CHCs (Kelstrup et al., 2014b; Oliveira et al., 2017; Oi et al., 2020). Thus, in this context, JH is likely to have the function of regulating chemical signals that are related to female fertility; hence, JH may contribute to the so-called

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'honest queen signal' (Kelstrup et al., 2014b; Oliveira et al., 2017; Oi et al., 2020).

JH mimics and compounds with anti-JH activity are important experimental tools for manipulating circulating JH levels and hence for understanding the physiological and behavioral roles of this important insect hormone (Slama, 1971; Ramaseshadri et al., 2012; Pandey et al., 2020). Precocene is a compound with anti-JH activity. It affects corpora allata size and, hence, JH production capacity, and can mediate the destruction of these glands (Bowers et al., 1976; Burns et al., 2007; Gotoh et al., 2008). In bumblebee workers, precocene-I can reduce the ovarian activation status and increase the proportion of ester in the Dufour gland secretion (Amsalem et al., 2014). In *Vespula vulgaris* wasps, however, precocene had no gonadotropic effect in workers but instead caused changes in their chemical profiles, making them less queen-like; in contrast, the JH mimic methoprene made the chemical profile of workers more queen-like (Oliveira et al., 2017). Previous studies have noted the importance of JH as an endocrine mediator in the lives of social insects, particularly relating to the division of labor, ovarian activity and chemical signaling (O'Donnell and Jeanne, 1993; Bloch et al., 2000; Sledge et al., 2004; Lengyel et al., 2007; Shorter and Tibbetts, 2009; Tibbetts et al., 2013; Kelstrup et al., 2014b; Amsalem et al., 2014; Norman and Hughes, 2016; Oliveira et al., 2017). Thus, JH might have acquired different functions to regulate social life during the evolution of social Hymenoptera (Hartfelder and Emlen, 2015).

The swarm-founding wasp *Polybia occidentalis* (Olivier 1791) (Vespidae: Polistinae, Epiponini) is an excellent model for studying how JH affects the division of labor. In this species, the organization of colonial work is fully partitioned among different teams of nestmates (O'Donnell and Jeanne, 1992). Brood care, foraging and handling of nest material are typical functions performed by workers (Jeanne, 1986; Jeanne et al., 1992), and the queen's task is to lay eggs. In addition, the workers can show flexibility by switching between tasks or by adjusting the rates of each task depending on colony needs (Jeanne et al., 1988).

In this study, we experimentally treated newly emerged *P. occidentalis* females with either methoprene or precocene-I to understand the role of JH in the ontogeny of worker activities. Our hypothesis is based on the premise that JH drives the progression of worker activities during their lives. Individuals treated with methoprene should begin activities earlier than expected and those treated with precocene should begin activities later. We also checked whether differences in the chemical composition of cuticular wax might reflect changes observed during different life stages of the workers. Finally, as methoprene might play a gonadotropic role by inducing a higher incidence of active ovaries in paper wasps (Giray et al., 2005; Shorter and Tibbetts, 2009), we investigated how JH mimics affect the reproductive status in workers of this species. Our results provide support for the hypothesis that JH (methoprene) and precocene have an effect on the cuticular chemical profile of workers associated with age polyethism. In addition, individuals treated with methoprene showed greater ovarian activation compared with that of individuals treated with solvent and precocene.

## MATERIALS AND METHODS

### Insect collection and treatments

This study was conducted in the Laboratório de Comportamento e Ecologia de Insetos Sociais at Universidade de São Paulo (USP), Ribeirão Preto Campus (21°09′50.7″S, 47°51′32.1″W) between October 2016 and May 2017. Colonies of *P. occidentalis* are relatively common in this area (da Silva et al., 2019). Combs containing pupae were removed from field colonies and kept inside plastic boxes in the laboratory to serve as a stock for newly emerged individuals. To decouple age, task and ovarian activation, we used the JH mimic methoprene and the anti-JH precocene. We used dosages leading to a relatively low mortality rate (based on pilot tests), allowing us to examine the effects of synthetic substances through most of the workers' lifespan. Newly emerged females were divided into four groups. Group I – methoprene-treated workers (MTW): females were treated topically on the dorsal part of the abdomen with a single 1 µl dose of 5 µg µl$^{-1}$ methoprene (Pestanal®, SUPELCO, analytical standard) diluted in acetone (69 individuals). Group II – precocene-treated workers (PTW): females received a single 1 µl topical application of 5 µg µl$^{-1}$ precocene-I (Sigma-Aldrich) diluted in acetone (71 individuals). Group III – solvent-treated workers (STW): females received a single dose of 2 µl of acetone (70 individuals). Group IV – non-treated workers (NTW): females received no treatment (69 individuals). Females from each group were paint-marked with non-toxic ink (Magic®) and then introduced into two experimental queenright post-emergent colonies located outside the laboratory for behavioral observations.

### Behavioral observations

We focused on three different functional groups of workers (Jeanne, 1986): cell inspectors (individuals walking on the outer comb performing cell checking); builders (workers repairing or building the nest envelope or combs); and foragers (workers arriving at the nest bringing in prey, pulp or liquid). The workers were collected and classified according to the behavior that was being performed at the moment of observation. To observe wasps performing cell inspection tasks, we experimentally removed part of the outer nest envelope. Behavioral observations were made from 09:00 h to 17:00 h for 10 min h$^{-1}$. During 10 min intervals, focal wasps were continuously filmed and then collected after performing determined behaviors (Altmann, 1974). Wasps were subsequently frozen (−20°C) for subsequent chemical analysis and ovary checking.

### CHC analysis

CHCs were extracted from individual wasps in a glass vial filled with hexane solvent (Macron Fine Chemicals, 95% *n*-hexane) for 2 min. The extracts were kept for 24 h in a flow chamber to evaporate the solvent. The chemical compounds were then resuspended in 50 µl of hexane, and 2 µl of this extract was injected (Splitless mode) in a gas chromatography system coupled with a mass spectrometer (GCMS; Shimadzu, model QP2010). We used a 30 m DB-5MS column, with helium gas flow set at 1 ml min$^{-1}$. The oven temperature began at 150°C and was ramped up by 7°C min$^{-1}$ until 260°C, and this temperature was maintained for 5 min. Next, the temperature was further raised to 310°C at a 5°C min$^{-1}$ ramping rate, where it was held for 10 min. The injector temperature was 280°C.

The software GCMS solutions for Windows (Shimadzu Corporation) was used to identify the respective compounds based on their mass spectra by comparison with the alkane standard solution $C_{21}$–$C_{40}$ (Fluka Analytical).

### Ovary analysis

The ovaries of 272 workers collected in the behavioral experiments were dissected in saline solution and categorized into two types: non-activated ovary (threadlike or filamentous ovarioles) and activated ovary (small nurse cells and oocytes, and few mature oocytes) (Fig. 1).

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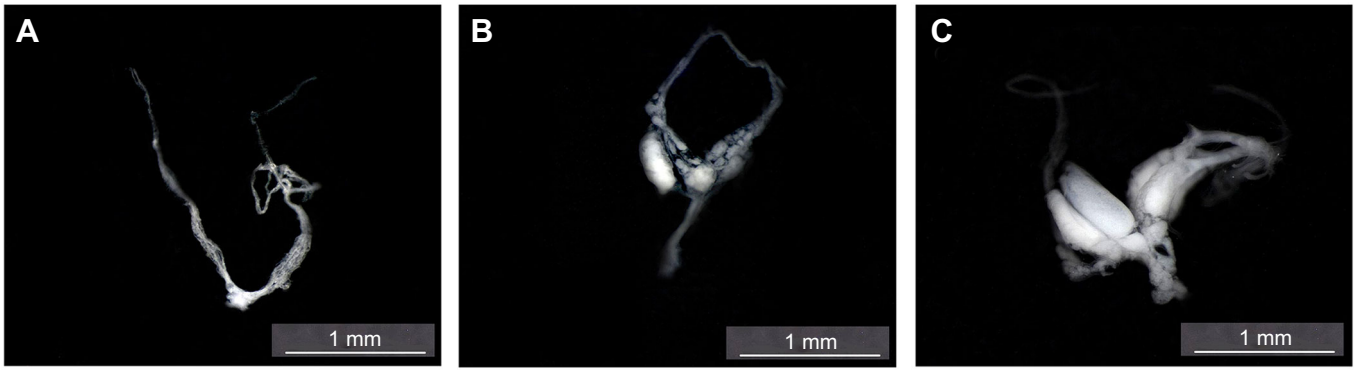


**Fig. 1. Ovarian activity patterns found in *Polybia occidentalis* workers.** Examples of ovaries showing (A) no activation or (B,C) activation.

### Statistical analyses

Generalized mixed models were run for the analysis of temporal polyethism concerning the treatments and tasks performed by the workers. We used age as the dependent variable, treatment/task as the explanatory variable, and comb source and colony (field) as random variables. The Akaike information criterion (AIC) was then used for selection of the best models. The residual diagnostics were analyzed (DHARMa package; https://cran.r-project.org/web/packages/DHARMa/index.html). A *post hoc* Tukey test was used to compare the means of the variables.

The CHC profiles were compared using the area percentage of each peak in the Permanova test (Bray–Curtis distance) to assess whether there were differences among groups in their chemical profiles. Compounds that contributed less than 0.5% to the total relative area were excluded from the statistical analysis. Non-metric multidimensional scaling (NMDS) analysis using Bray–Curtis distances was performed to spatially visualize the chemical data. A cluster analysis was conducted in R software to identify the similarity and dissimilarity in the chemical compounds among the tasks and treatments. The mean value for each compound (log-transformed) was calculated and used to make graphs. A discriminant analysis (SIMPER) was used to infer which compounds contributed the most to the separation of the groups (significant *P*-value). This analysis compares each compound between the treatment groups and gives the *P*-value of this contrast.

Generalized mixed models were run to analyze the ovarian status of the workers between the treatments and tasks. Ovary was the dependent variable, treatment/task was the explanatory variable, and comb source and colony (field) were random variables, using a binomial distribution. AIC was then used for selection of the best models. A *post hoc* Tukey test was used to compare the means of the variables. All statistical analyses were run in the R program environment, using the lme4 package (http://www.R-project.org/; https://cran.r-project.org/web/packages/lme4/index.html).

## RESULTS

### Behavioral patterns

#### Age polyethism within each treatment

Among workers of the STW group, the cell inspectors and foragers showed significantly different patterns in terms of the timing of task performance (mean±s.d.: 8±5 versus 12±4 days, respectively; Tukey test: $z$=3.224, $P$<0.01). The initiation of building coincided with the onset of foraging; thus, these wasps differed significantly in age from the cell inspectors (12±4 days; Tukey test: $z$=−2.998, $P$<0.01) (Fig. 2B). The age of builders and foragers did not differ (Tukey test: $z$=0.027, $P$=0.9787).

Workers of the MTW group performed cell inspection activities 5 days after introduction into the colony (5±3 days). Building behavior was observed after 11 days (11±3 days), and foraging was initiated at a mean of 13 days (13±4 days). The temporal pattern of task performance significantly differed concerning the onset of cell inspection versus building (Tukey test: $z$=−4.533, $P$<0.01), cell inspection versus foraging (Tukey test: $z$=6.615, $P$<0.01), and building versus foraging (Tukey test: $z$=2.239, $P$=0.0252) (Fig. 2C).

Cell inspection activity in the PTW group began at the age of 9 days (9±11 days). Building behavior also began after 9 days (9±2 days), but foraging was observed only after 13 days (13±4 days). Statistical differences were not observed between the onset of cell inspection and building (Tukey test: $z$=−0.157, $P$=0.8754) and, cell inspection and foraging (Tukey test: $z$=1.865, $P$=0.0933), but did differ between foraging and building (Tukey test: $z$=4.407, $P$<0.01) (Fig. 2D).

#### Age polyethism within each task

The comparison of the age among the treatments for each activity revealed that cell inspectors showed significant differences for MTW×STW (Tukey test: $z$=−2.47, $P$<0.01) and MTW×PTW (Tukey test: $z$=3.66, $P$<0.01) (Fig. 3A; Table S1). With respect to the building task, a significant difference was observed for STW×PTW (Tukey test: $z$=−2.42, $P$=0.04) (Fig. 3B; Table S1). For the foraging task, the group that differed was NTW×STW (Tukey test: $z$=3.55, $P$<0.01) (Fig. 3C; Table S1).

### Relationship between hormonal treatment and CHC profile

#### Comparison of CHCs within each treatment

The CHC profiles of workers (Table S2) treated with the JH analog methoprene or the anti-JH compound precocene-I were significantly affected. The CHC profile followed changes linked with age polyethism (Fig. 4). Based on the permutation analysis, the MTW (Permanova: $F_{2,66}$: 16.365, $P$<0.01) and PTW (Permanova: $F_{2,68}$: 4.2925, $P$<0.01) groups showed specific profiles related to age polyethism. The STW group (Permanova: $F_{2,67}$: 5.9051, $P$<0.01) also differed in the chemical profiles of workers performing certain tasks.

There were specific compounds that were important for task separation and that were shared between the STW and MTW group. In the cell inspector×builder comparison, the common compounds were 13-;11-;9-$MeC_{27}$, 15-;14-;13-;12-;11-;10-$MeC_{30}$, 15-;13-;11-$MeC_{39}$, 5-$MeC_{27}$ and 5-$MeC_{29}$. For the comparison cell inspector×forager, the shared compounds between groups were 3.13-;3.11di-$MeC_{29}$, 13-;11-;9-$MeC_{27}$, 15-;13-;11-$MeC_{39}$ and 5-$MeC_{27}$. However, when builder and forager were compared,

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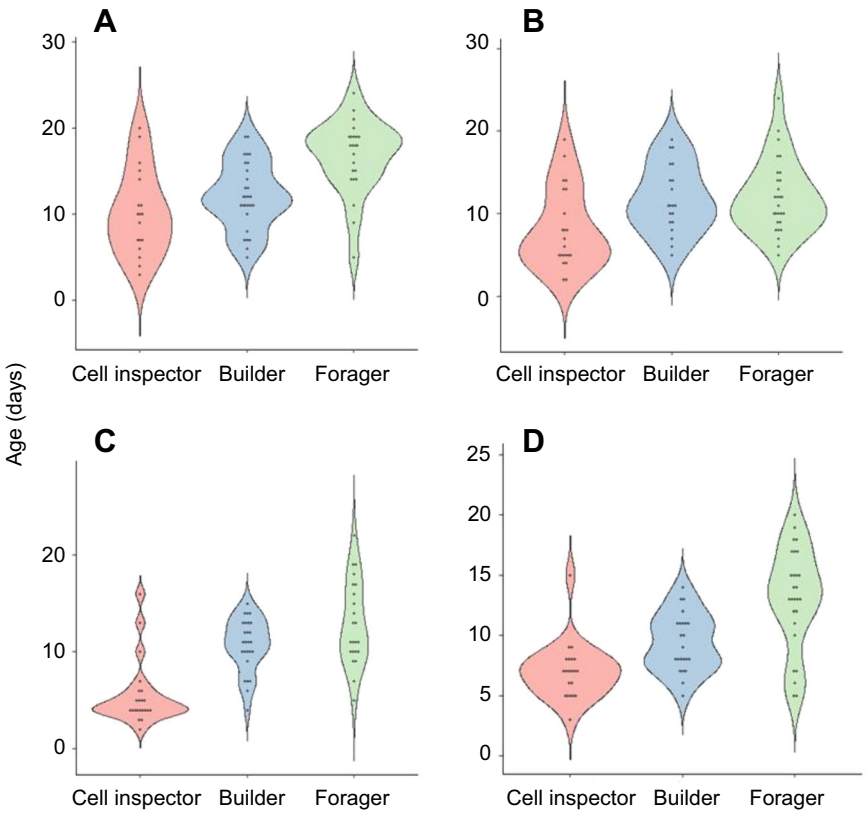


**Fig. 2. Age distribution of workers performing each task according to treatment.** Tasks were classified as cell inspector, builder or forager for workers in the four treatment groups: (A) non-treated workers (NTW; 69 individuals); (B) solvent-treated workers (STW; 70 individuals); (C) methoprene-treated workers (MTW; 71 individuals); and (D) precocene-treated workers (PTW; 71 individuals). Violin plots of individual data (dots). NTW: cell inspector×forager (Tukey test) $z$=4.825, $P$<0.01; cell inspector×builder $z$=−1.684, $P$=0.092; builder×forager $z$=3.604, $P$<0.01. STW: cell inspector×forager $z$=3.224, $P$<0.01; cell inspector×builder $z$=−2.998, $P$<0.01; builder×forager $z$=0.027, $P$=0.9787. MTW: cell inspector×forager $z$=6.615, $P$<0.01; cell inspector×builder $z$=−4.533, $P$<0.01; builder×forager $z$=2.239, $P$=0.0252. PTW: cell inspector×forager $z$=1.865, $P$=0.0933; cell inspector×builder $z$=−0.157, $P$=0.8754; builder×forager $z$=4.407, $P$<0.01.

there were not any common compounds between STW and MTW groups (Table S3).

In the STW group, the branched methyl alkanes 13-;11-;9-$MeC_{27}$ and 15-;13-;11-$MeC_{39}$ appeared in greater proportions in the cell inspectors than in the builders, whereas the compounds 5-$MeC_{27}$ and 5-$MeC_{29}$ were higher in the builders (Table 1). The same pattern was maintained in the MTW group. These compounds (13-;11-;9-$MeC_{27}$ and 15-;13-;11-$MeC_{39}$) were proportionally higher in cell inspectors than in foragers, and 5-$MeC_{27}$ was higher in forages, in both the STW and MTW groups (Table 1). Only the compounds 15-;14-;13-;12-;11-;10-$MeC_{30}$ and 3.13-;3.11di-$MeC_{29}$ were different between groups. These two compounds showed higher proportions in cell inspectors in the STW group, but in the MTW group a different pattern was noticed, with greater expression in the builders (15-;14-;13-;12-;11-;10-$MeC_{30}$) and foragers (3.13-; 3.11di-$MeC_{29}$) (Table 1).

A common compound between STW and PTW was seen only for cell inspector×builder (15-;13-;11-$MeC_{39}$) and this compound appeared in a higher proportion in the cell inspector (in both treatment groups) (Table 1). In contrast, in the MTW and PTW comparison, shared compounds were present for cell inspector×builder (5.15-;5.13-;5.11di-$MeC_{29}$ and 15-;13-;11-$MeC_{39}$) and cell inspector×forager (5.15-;5.13-;5.11di-$MeC_{29}$ and 5-$MeC_{29}$) (Table 1). In the first comparison, the dimethyl compound was present in a higher proportion in the builder than in the cell inspector, and the methyl compound in a higher proportion in the cell inspector, in both treatment groups (Table 1). In the cell

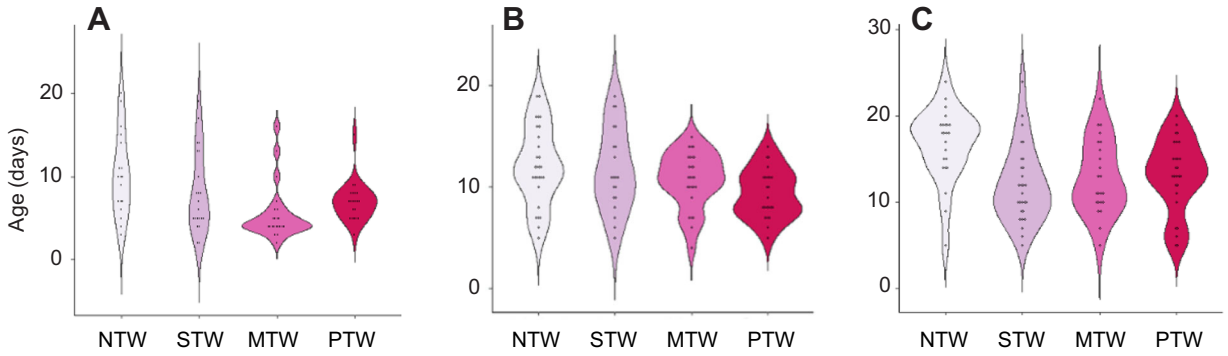


**Fig. 3. Age distribution of workers in each treatment group according to task.** (A) Cell inspector (83 individuals); (B) builder (97 individuals) and (C) forager (101 individuals). Cell inspector: NTW×STW (Tukey test) $z$=1.468, $P$=0.1999; NTW×MTW $z$=3.803, $P$<0.01; NTW×PTW $z$=−0.008, $P$=0.9935; STW×MTW $z$=−2.474, $P$=0.0267; STW×PTW $z$=1.383, $P$=0.1999; MTW×PTW $z$=3.669, $P$<0.01. Builder: NTW×STW $z$=0.605, $P$=0.5448; NTW×MTW $z$=2.073, $P$=0.0764; NTW×PTW $z$=−3.273, $P$<0.01; STW×MTW $z$=−1.295, $P$=0.2601; STW×PTW $z$=−2.423, $P$=0.0461; MTW×PTW $z$=−1.235, $P$=0.2601. Forager: NTW×STW $z$=3.553, $P$<0.01; NTW×MTW $z$=3.047, $P$<0.01; NTW×PTW $z$=−2.574, $P$=0.02008; STW×MTW $z$=0.395, $P$=0.6926; STW×PTW $z$=1.069, $P$=0.4278; MTW×PTW $z$=0.622, $P$=0.6410.

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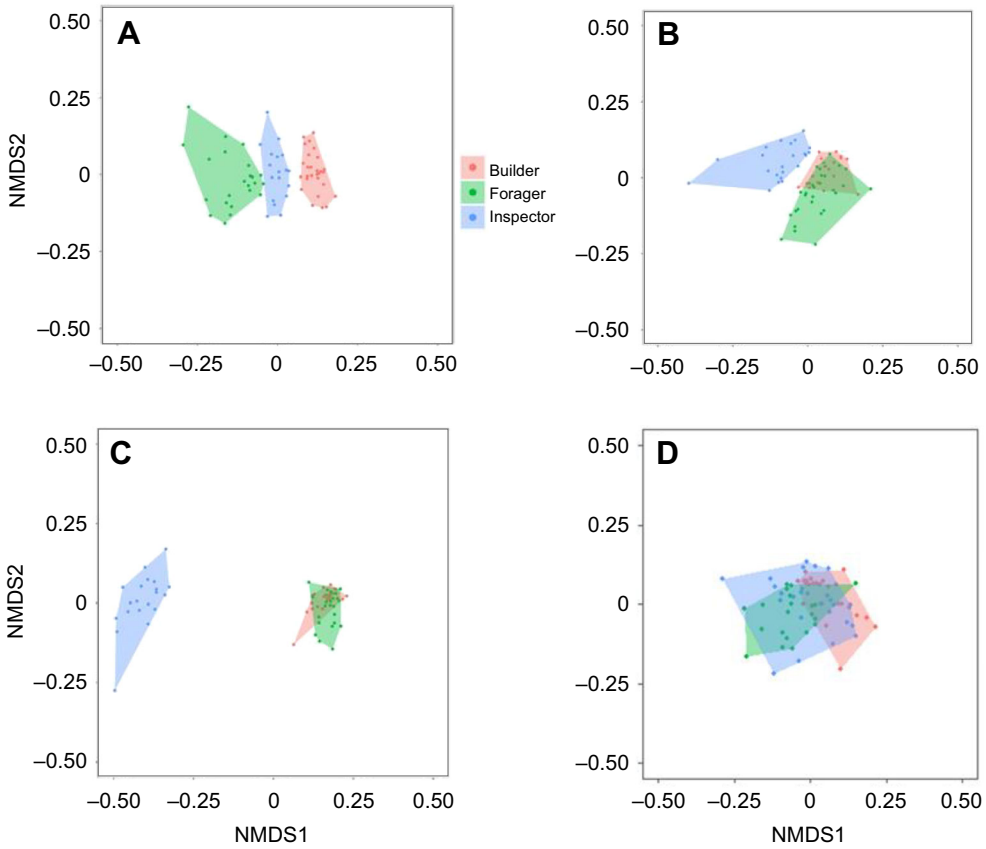


**Fig. 4. Cuticular hydrocarbon (CHC) profile of workers in each treatment group according to task.** Non-metric multidimensional scaling (NMDS) analysis of CHCs in (A) NTW (69 individuals, stress value - 0.15); (B) STW (70 individuals, stress value - 0.18); (C) MTW (69 individuals, stress value - 0.10) and (D) PTW (71 individuals, stress value - 0.20) groups.

inspector×forager comparison, the compounds were present in a greater quantity in the forager task in both treatment groups (Table 1).

### Comparison of CHCs within each task

The chemical profiles of workers performing each task were compared between the treatments. For the cell inspection task, the MTW, PTW and STW groups differed significantly (Permanova: $F_{3,77}$=8.9119, $P$<0.01; Table S4). When comparing workers performing building tasks from MTW, PTW and STW groups (Permanova: $F_{3,93}$=3.7563, $P$<0.01), chemical variation was not observed: STW×MTW $P$=0.224, STW×PTW $P$=0.201 and MTW×PTW $P$=0.350. CHCs of foragers showed clear differences among all groups (STW, MTW and PTW; Permanova: $F_{3,97}$=3.4277, $P$<0.01), except STW×PTW ($P$=0.157) (Table S4).

**Table 1. Important chemical compounds shared by workers in the different treatment groups according to task**

| | Cell inspector | Builder | Forager |
|---|---|---|---|
| STW | | | |
| 13-;11-;9-MeC$_{27}$ | 2.360±0.891 | 1.708±0.496 | 1.842±0.436 |
| 5-MeC$_{27}$ | 0 | 0.574±0.176 | 0.565±0.149 |
| 5-MeC$_{29}$ | 1.25±0.188 | 1.486±0.302 | 1.256±0.163 |
| 3.13-;3.11di-MeC$_{29}$ | 5.473±1.192 | 5.644±0.749 | 5.011±1.063 |
| 15-;14-;13-;12-;11-;10-MeC$_{30}$ | 3.804±0.502 | 4.245±0.497 | 3.945±0.339 |
| 15-;13-;11-MeC$_{39}$ | 0.631±0.316 | 0 | 0 |
| MTW | | | |
| 13-;11-;9-MeC$_{27}$ | 2.974±1.324 | 1.703±0.385 | 2.021±0.565 |
| 5-MeC$_{27}$ | 0 | 0.543±0.150 | 0.674±0.325 |
| 5-MeC$_{29}$ | 1.019±0.245 | 1.457±0.184 | 1.317±0.236 |
| 5.15-;5.13-;5.11di-MeC$_{29}$ | 0 | 0.698±0.263 | 0.814±0.262 |
| 3.13-;3.11di-MeC$_{29}$ | 4.545±1.037 | 5.519±0.876 | 5.763±0.827 |
| 15-;14-;13-;12-;11-;10-MeC$_{30}$ | 3.457±0.568 | 4.271±0.331 | 3.888±0.478 |
| 15-;13-;11-MeC$_{39}$ | 0.568±0.231 | 0 | 0 |
| PTW | | | |
| 5-MeC$_{29}$ | 1.304±0.190 | 1.363±0.101 | 1.307±0.285 |
| 5.15-;5.13-;5.11di-MeC$_{29}$ | 0 | 0.643±0.357 | 0.697±0.364 |
| 15-;13-;11-MeC$_{39}$ | 0.621±0.321 | 0 | 0.569±0.423 |

STW, solvent-treated workers; MTW, methoprene-treated workers; PTW, precocene-treated workers. Data (relative percentages) are means±s.d.

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**Table 2. Important chemical compounds shared by workers performing different tasks according to treatment group**

| | STW | MTW | PTW |
|---|---|---|---|
| Cell inspector | | | |
| 3-MeC$_{27}$ | 2.217±0.491 | 1.421±0.496 | 2.229±0.646 |
| *n*-C28 | 1.378±0.248 | 1.156±0.191 | 1.375±0.166 |
| 5-MeC$_{29}$ | 1.25±0.188 | 1.019±0.245 | 1.304±0.190 |
| 3-MeC$_{29}$ | 7.410±1.127 | 6.023±1.298 | 7.435±0.957 |
| 3.13;3.11di-MeC$_{29}$ | 5.473±1.192 | 4.545±1.037 | 4.942±1.039 |
| 5.15-;5.13-;5.11di-MeC31 | 0.879±0.211 | 0.696±0.257 | 0.74±0.154 |
| Builder | | | |
| 5-MeC$_{29}$ | 1.486±0.302 | 1.457±0.184 | 1.363±0.101 |
| 3-MeC$_{29}$ | 8.636±0.650 | 8.022±0.796 | 8.088±0.506 |
| Forager | | | |
| 3-MeC$_{27}$ | 2.599±0.821 | 3.073±0.824 | 2.440±0.687 |
| *n*-C28 | 1.583±0.295 | 1.389±0.265 | 1.442±0.198 |
| 3.13;3.11di-MeC$_{29}$ | 5.011±1.063 | 5.763±0.827 | 4.790±0.764 |
| 5.15-;5.13-;5.11di-MeC31 | 0.772±0.185 | 0.84±0.191 | 0.784±0.183 |

Data (relative percentages) are means±s.d.

Specifically, there were compounds that were important for distinguishing workers from different treatments, and that were shared between workers performing cell inspection and building tasks. In the STW×MTW comparison, the common compounds were 3-MeC$_{29}$ and 5-MeC$_{29}$. These specific compounds appeared in higher quantity in the STW group in both tasks. The other treatment comparisons (STW×PTW and MTW×PTW) lacked shared chemical compounds (Table S5).

Furthermore, some compounds were present in workers performing cell inspection and foraging tasks. Comparing the STW×MTW groups, the common compounds were 3-MeC$_{27}$, *n*-C$_{28}$, 3.13;3.11di-MeC$_{29}$, and 5.15-;5.13-;5.11di-MeC$_{31}$. The alkane *n*-C$_{28}$ was higher in the STW group and 3.13;3.11di-MeC$_{29}$ in the MTW group in both tasks (cell inspection and foraging). However, 3-MeC$_{27}$ and 5.15-;5.13-;5.11di-MeC$_{31}$ were higher in workers performing cell inspection from the STW group, but in workers displaying the foraging task these compounds were overexpressed in the MTW group (Table 2). Only the compound 3-MeC$_{27}$ was common for MTW×PTW comparisons. Workers performing cell inspection from the PTW group and workers performing foraging activities from the MTW group had a higher abundance of 3-MeC$_{27}$. No compound was common to STW×PTW groups (Table 2). Workers displaying building and foraging tasks shared common compounds only between the MTW and PTW groups. In this case, the chemical compound in common was 3.13;3.11di-MeC$_{29}$ and it was higher in females displaying both tasks from the MTW (Table 2).

## Ovary activation

### Comparison of ovary activation within each treatment

Analysis of the ovaries of workers from the NTW group showed that 89.9% had non-activated ovaries and 10.1% had activated ovaries. A total of 83.6% of STW had non-activated ovaries, and 16.4% had activated ovaries. Of the MTW group, 45.5% had ovaries with no activation and 54.5% had activated ovaries. For the PTW group, 78.3% had non-activated ovaries and 21.7% had activated ovaries. Overall, we noticed a positive trend of the MTW group (all tasks together) showing the highest rate of females with activated ovaries, whereas we noticed the opposite trend for the PTW group (all tasks together), which expressed the lowest rates of females with ovary activation.

We did not observe significant differences between tasks within each treatment, with the exception of the PTW group, for workers displaying the cell inspection×foraging task (Tukey test: $z=-2.73$, $P=0.01$) (Table 3). This means that overall females performing different tasks (cell inspection, building and foraging) in each of the treatments (STW, MTW and PTW) expressed similar patterns of ovary activation.

When comparing between treatments, we observed that females from the NTW and STW groups expressed equal rates of activated ovaries and non-activated ovaries (Tukey test: $z=-1.140$, $P=0.3053$). The analysis between the STW and MTW groups revealed significant differences between the levels of ovary activation (Tukey test: $z=4.398$, $P<0.01$), with females from the MTW group expressing higher rates of ovary activation. The PTW group did not differ from the STW group, as the frequency of activated and non-activated ovaries was similar (Tukey test: $z=0.728$, $P=0.4665$). Finally, we detected significant differences in levels of ovary activation between females from the MTW and PTW groups, with the MTW group expressing more activated ovaries, whereas the PTW group expressed more non-activated ovaries (Tukey test: $z=-3.888$, $P<0.01$).

### Comparison of ovary activation among treatments in each task

The proportion of ovary activation in workers varied according to the different treatments in each task. In cell inspectors, we verified that there were no significant differences between types of ovaries among the treatments for the MTW, PTW and STW group (Fig. 5A;

**Table 3. Comparison of ovary types of workers performing different tasks according to treatment group**

| | *P* | *z* |
|---|---|---|
| NTW | | |
| Cell inspector×builder | 0.511 | 1.168 |
| Cell inspector×forager | 0.511 | −0.953 |
| Builder×forager | 0.844 | 0.197 |
| STW | | |
| Cell inspector×builder | 0.106 | 1.95 |
| Cell inspector×forager | 0.106 | −1.806 |
| Builder×forager | 0.498 | 0.678 |
| MTW | | |
| Cell inspector×builder | 0.82 | 0.402 |
| Cell inspector×forager | 0.82 | 0.61 |
| Builder×forager | 0.82 | 0.218 |
| PTW | | |
| Cell inspector×builder | 0.15 | 1.439 |
| Cell inspector×forager | 0.019* | −2.726 |
| Builder×forager | 0.15 | −1.546 |

*Significant *P*-values (Tukey test).

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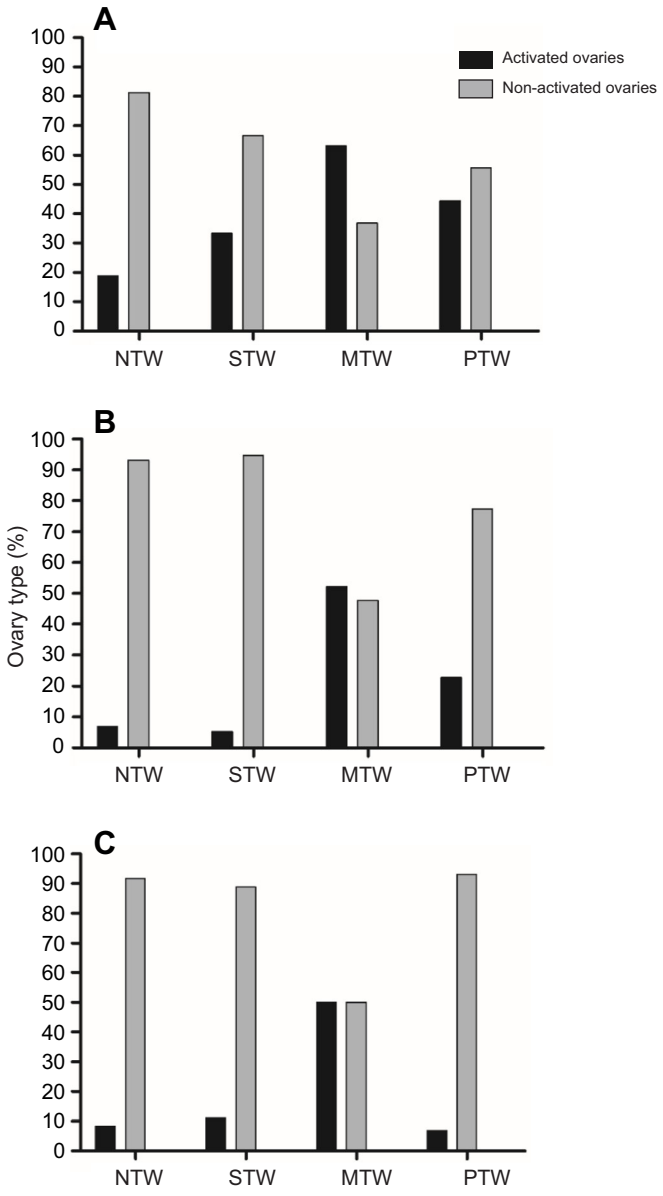


**Fig. 5. Percentage of each ovary type for workers in each treatment group according to task.** Ovaries were classified as activated or non-activated for workers performing the three tasks: (A) Cell inspector (74 individuals), (B) builder (93 individuals) and (C) forager (104 individuals).

Table S6). In the builder group, STW females had more non-activated ovaries when compared with MTW females (Tukey test: $z$=2.685, $P$=0.0217), but STW females and PTW females expressed similar levels of ovary activation (Tukey test $z$=1.454, $P$=0.1752). Females performing building tasks from the MTW and PTW groups did not differ in terms of ovary activation (Tukey test $z$=−1.992, $P$=0.0927; Fig. 5B; Table S6). Lastly, for the forager group, we observed significant differences between the types of ovaries expressed in females from the STW and MTW groups (Tukey test $z$=2.831, $P$<0.01) and between females from the MTW and PTW groups (Tukey test $z$=−3.122, $P$<0.01); overall, females belonging to the MTW group had the lowest proportion of non-activated ovaries when compared with the other groups. Females of the PTW group did not differ from females from the STW group in terms of activated and non-activated ovaries (Tukey test $z$=−0.558, $P$=0.8465) (Fig. 5C; Table S6).

## DISCUSSION

In this study, we provide strong support for the hypothesis that the cuticular chemical profile is connected with the activities displayed by workers, which is regulated by different circulating levels of JH. In addition, our data demonstrate that JH acts as a gonadotropic hormone in *P. occidentalis* workers. The effects of methoprene treatment on the transition between tasks were not clear when compared with the STW group. Acetone (STW group) has a known toxic effect at the cellular level (Lengyel et al., 2007), which might have potentially affected the normal cycle of treated workers. This effect of the acetone treatment could be mediated by the pre-existing JH, as this hormone is sensitive to stress in many insects (Jankovic-Hladni, 1991). Even so, we found that females started displaying the cell inspection task significantly earlier when treated with methoprene than when treated with acetone. Furthermore, we observed that the anti-JH compound precocene-I had no evident effect on the dynamics of task performance, except in the building task, but not as expected, as females performing the building task that had been treated with precocene were younger than those that had been treated with acetone. O'Donnell and Jeanne (1993) showed previously the effect of the JH on the transition, but here we present further evidence that JH plays a gonadotropic role and can modify CHC expression in *P. occidentalis* workers.

### Behavioral patterns

We observed significant effects related to task progression compared with the STW group only in the cell inspection task. This result was expected considering that methoprene acts as a JH analog (O'Donnell and Jeanne, 1993), and increases in the endogenous JH titer have been shown to affect the transition between tasks in several highly eusocial Hymenoptera (Robinson, 1987; Huang and Robinson, 1992; Hartfelder, 2000; Kelstrup et al., 2014b). In ants, social bees and social wasps, the topical application of JH analog is known to affect forager tasks and lead to an earlier age of onset for foraging (Robinson, 1987; Schulz et al., 2002; Shorter and Tibbetts, 2009; Norman and Hughes, 2016).

Precocene treatment had an effect on the regulation of behavior only between the building and foraging tasks. Precocene is an anti-JH compound that diminishes or abolishes JH production by the insect corpora allata (Bowers et al., 1976; Burns et al., 2002, 2007; Amsalem et al., 2014). However, our hypothesis that the *P. occidentalis* workers receiving this treatment would start their activities later than the wasps treated with solvent was not confirmed.

### Ovary activation

The frequently postulated and asserted gonadotropic role of JH in social insects is explained by several observations. In *P. occidentalis* workers, we found that the MTW group had a higher proportion of workers with activated ovaries than did the STW and PTW groups. Even among the tasks, the highest rate of ovarian activation was observed in the MTW group. However, the ovaries of the MTW females were still quite different from queen-like ovaries, which typically contain mature oocytes ready to be laid (Noll and Zucchi, 2000). The activation of *P. occidentalis* ovaries has previously been reported to decrease with age and when they begin to work outside the nest (O'Donnell, 2001), but we did not find a significant difference between workers performing the different tasks. However, O'Donnell (2001) suggested that worker polyethism could be independent of reproductive physiology in advanced eusocial species. In contrast with our results, studies in some social wasps and honeybees have shown that JH treatment did not

influence the ovarian activation of workers compared with individuals that did not receive the hormone (Robinson et al., 1991; Kelstrup et al., 2014a).

Precocene-I exposure also did not negatively affect worker ovarian status compared with the STW group. Nonetheless, our results are similar to those observed in *V. vulgaris* workers, where precocene treatment also had no gonadotropic effects (Oliveira et al., 2017). These negative results in terms of ovary status might stem from rapid metabolization of the toxic precocene by the hemolymph and/or various tissues (e.g. the fat body and gut) observed in some holometabolous insects, which prevents it from reaching the corpora allata (Ohta et al., 1977; Burt et al., 1978). Another hypothesis suggests that precocene is not bioactive itself and needs to be converted to an activated metabolite (Bergot et al., 1980). We do not have information on the sensitivity of *P. occidentalis* to precocene. More studies analyzing hemolymph titer and corpora allata activity are necessary. Our results are in contrast with results previously reported in bumblebees, in which precocene-I had no effect on the timing of nursing and foraging activities but was significantly correlated with the ovarian status of treated individuals (Röseler, 1977; Shpigler et al., 2016; Pandey et al., 2020).

Two hypotheses have been proposed to explain the role of the JH in regulating the social life of Hymenoptera and specifically its effect on ovarian activation and/or age polyethism. The first is the 'novel-function' hypothesis, in which JH only has a single role (either ovarian activation or age polyethism) in highly social insects (Robinson, 1992; West-Eberhard, 1996; Robinson and Vargo, 1997). The second is the 'split-function' hypothesis, which posits that JH ancestral functions play roles in both gonadotropic and behavioral development in the same species (Robinson, 1992; West-Eberhard, 1996; Robinson and Vargo, 1997). The second hypothesis predicts that JH affects different members of the colony differently depending on their level of nourishment. Queens are well-nourished during the larval and adult phases; consequently, they achieve complete ovary activation. This process is regulated by JH, which controls oogenesis and egg laying. In contrast, workers are poorly nourished during the development phases, and this results in the reduced activation of ovaries (Robinson, 1992; West-Eberhard, 1996; Robinson and Vargo, 1997).

Our results also support the split-function hypothesis, but with some caveats. We found that JH operates in both aspects (behavioral and ovarian activation). These effects can be linked with the reproductive capacity that has been observed in workers of *P. occidentalis* (mainly in young females) (O'Donnell, 2001). Furthermore, the hormonal mechanism that regulates behavioral development is connected with the mechanism responsible for coordinating the reproductive physiology of ancestral species (West-Eberhard, 1996; Robinson and Vargo, 1997; Tibbetts et al., 2013). Thus, rather than being decoupled as in *Apis mellifera* (Robinson, 1992), these mechanisms continue to act together to control both processes regardless of caste in this species; the workers may eventually become queens during colonial ontogeny. Observations of social Hymenoptera support the split-function hypothesis; for example, in *Polistes canadensis* and *Polistes dominula*, JH has a dual function as both a gonadotropic and behavior regulator (Giray et al., 2005; Shorter and Tibbetts, 2009; Tibbetts et al., 2018).

### Relationship between hormonal treatment and CHC profile

The clear effect of JH (methoprene) observed on *P. occidentalis* workers was related to the expression of CHCs and their association with age polyethism. The modification observed in the chemical profile followed the shifts in age polyethism, and this concordance is important because the workers in a colony receive, decode and process information about the individual states of their nestmates, as well as the needs of the colony (Wyatt, 2003; Howard and Blomquist, 2005). Specifically, we found that the MTW group showed a higher number of important compounds separating the tasks when compared with the STW and PTW groups. The complexity of the compounds was also high in the MTW group compared with the others. Previous studies detected a genetic pathway linking JH action to CHC biosynthesis, in which individuals that were treated with a JH mimic tended to overproduce long-chain hydrocarbons (mainly for $C_{27}$ to $C_{31}$ carbon atoms) (Morgan, 2010; Blomquist, 2010). The JH analog acts on the chain stretching of the compounds (fatty acid) that is the precursor of CHC (Morgan, 2010; Blomquist, 2010). Although methoprene treatment may affect CHC production, the effect of the task on the compounds is clear, given that each task had a specific chemical profile within the treatment groups. Comparing across the same tasks, some compounds did not change proportion with methoprene treatment (e.g. 13,11,9-Me$C_{27}$ in cell inspector task). This pattern would suggest that these compounds can be important to task distinction. Previous studies in social wasp species suggested that the linear alkanes and methyl alkane groups act as important cues for both nestmate recognition and caste differentiation (Bonavita-Cougourdan et al., 1991; Dani et al., 2001; Sledge et al., 2001; Tannure-Nascimento et al., 2007; Van Oystaeyen et al., 2014; Oi et al., 2015b). Furthermore, our data showed that methoprene affected the CHCs expressed within each task. In workers performing the cell inspection and foraging tasks, the chemical profiles were different between all treatments, which provides evidence that variation in JH titer may directly affect CHC production. Similar results were observed in *Synoeca surinama*, where methoprene treatment affected the CHC profile of females independently of their gonadotropic status (Kelstrup et al., 2014b). However, the MTW group performing building tasks in our work did not differ in their CHC profiles from those in the STW group. In this case, the solvent may have had an unexpected effect on the chemical profile. Previous studies have shown that treatment with solvent (pentane) on the cuticle of the ant *Camponotus vagus* resulted in a reduction in its endogenous hydrocarbon level within 3 h of application (Meskali et al., 1995). Furthermore, building behavior is flexible and is driven by demand according to the level of damage to the nest (Jeanne, 1996). The colony even has a supply of females that can promptly replace others in building activities as necessary (Jeanne, 1996), so this system probably has an impact on the chemical profile expressed.

In addition, precocene (PTW group) may have affected hydrocarbon synthesis. As previously mentioned, precocene can be metabolized in internal tissues such as the fat body (Ohta et al., 1977; Burt et al., 1978). One of the sources of hydrocarbon synthesis is the fat body cells (oenocytes), and previous work has shown that the internal hydrocarbons are qualitatively similar to cuticular hydrocarbons (Schal et al., 1998; Bagnères and Blomquist, 2010). Specifically, internal hydrocarbons have been suggested to be represented by a pool of hydrocarbons in the oenocytes, hemolymph, epidermis and fat bodies (Schal et al., 1998). Thus, these compounds resulting from the process of metabolism could be used to synthesize new chemical products.

Overall, our CHC data for *P. occidentalis* are consistent with the tasks that the workers performed, suggesting that there is a robust link between these traits. Such a connection may be a common feature of social Hymenoptera, given that past studies have shown

that the chemical cuticular profile of *A. mellifera* workers depends on the innate conditions that are associated with age polyethism of individuals (Vernier et al., 2019). We found that the CHC compounds varied both quantitatively and qualitatively in *P. occidentalis* workers, but some important compounds responsible for the chemical differentiation of the workers were shared among the different tasks and their treatments. This variation is likely important for chemical communication among nestmates, as these cues permit wasps to identify which tasks individuals are performing, thus avoiding overlap in worker task performance during colony development (Blomquist et al., 1987). A relationship between CHC profiles and age polyethism has been reported for several social insects in addition to *P. occidentalis*, including honeybees and ants, in which different chemical profiles distinguish between functional worker castes (Martin and Drijfhout, 2009; Ferreira-Caliman et al., 2010; Kather et al., 2011; Valadares and Nascimento, 2016; Balbuena et al., 2018).

## Conclusion

In sum, the results of our experimental manipulation of JH titer in the swarming social wasp *P. occidentalis* support the hypothesis that endogenous JH levels regulate CHC expression, which is linked to age polyethism in workers (O'Donnell and Jeanne, 1993). JH also appears to affect, albeit moderately, ovarian activity of *P. occidentalis* workers; these findings differ from results obtained for other species, such as *Polybia micans* and *A. mellifera*, where JH has no gonadotropic effect in workers, and are similar to observations made in the epiponine wasp *S. surinama* (Fluri et al., 1981; Robinson and Vargo, 1997; Kelstrup et al., 2014a,b). Our results provide additional data supporting the view that the ancestral gonadotropic role of JH has undergone several modifications in social Hymenoptera and that it has been maintained in some lineages but not others. In addition, the association of cuticular chemical profiles with communication in eusocial Hymenoptera appears to be a rather conserved link. This conclusion is based on the fact that CHC production capacity and their use in some interactions have been documented in both solitary and parasitic insects (Kather and Martin, 2015). This observation provides evidence of the importance of task- and age-specific CHC profiles for the maintenance and cohesion of the colony life cycle.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: A.P., F.S.d.N.; Methodology: A.P., S.M., F.S.d.N.; Formal analysis: A.P., R.C.d.S., D.S.A., S.M.; Investigation: A.P., S.M.; Data curation: A.P., R.C.d.S., D.S.A.; Writing - original draft: A.P., F.S.d.N.; Writing - review & editing: A.P., R.C.d.S., D.S.A., S.M., K.H., F.S.d.N.; Supervision: K.H., F.S.d.N.

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**Table S1.** Comparison of age variation in the tasks between the treatments. * Significant p-values.

| Cell inspector | Tukey test | p | Builder | Tukey test | p | Forager | Tukey test | p |
|---|---|---|---|---|---|---|---|---|
| NTW x STW | 1.468 | 0.1999 | NTW x STW | 0.605 | 0.5448 | NTW x STW | 3.553 | <0.01* |
| NTW x MTW | 3.803 | <0.01* | NTW x MTW | 2.073 | 0.0764 | NTW x MTW | 3.047 | <0.01* |
| NTW x PTW | -0.008 | 0.9935 | NTW x PTW | -3.273 | <0.01* | NTW x PTW | -2.574 | 0.02008* |
| STW x MTW | -2.474 | 0.0267* | STW x MTW | -1.295 | 0.2601 | STW x MTW | 0.395 | 0.6926 |
| STW x PTW | 1.383 | 0.1999 | STW x PTW | -2.423 | 0.0461* | STW x PTW | 1.069 | 0.4278 |
| MTW x PTW | 3.669 | <0.01* | MTW x PTW | -1.235 | 0.2601 | MTW x PTW | 0.622 | 0.6410 |

**Table S2.** Chemical compounds profile from *P. occidentalis* workers of all treatments with peak number, retention time (min) and compounds name.

| Peaks | Retention time (min) | Compounds | Peaks | Retention time (min) | Compounds |
|---|---|---|---|---|---|
| 1 | 10.530 | z-$C_{21}$ | 37 | 23.660 | 15-;14-;13-;12-;11-;10Me$C_{30}$ |
| 2 | 10.711 | n-$C_{21}$ | 38 | 24.255 | 4Me$C_{30}$ |
| 3 | 11.940 | n-$C_{22}$ | 39 | 24.517 | 3Me$C_{30}$ |
| 4 | 13.002 | z-$C_{23}$ | 40 | 24.606 | z-C31 |
| 5 | 13.025 | n-$C_{23}$ | 41 | 24.754 | 4,16-;4,14-;4,12 diMe$C_{30}$ |
| 6 | 14.277 | n-$C_{24}$ | 42 | 24.809 | n-$C_{31}$ |
| 7 | 15.391 | n-$C_{25}$ | 43 | 24.822 | 3,15-; 3,13-;3,11diMetilC$_{30}$ |
| 8 | 15.769 | 13-;11-;9Me$C_{25}$ | 44 | 25.390 | 15-;13-;11-;9-;7Me$C_{31}$ |
| 9 | 16.210 | 5Me$C_{25}$ | 45 | 25.645 | 7Me$C_{31}$ |
| 10 | 16.211 | 3Me$C_{25}$ | 46 | 25.770 | 5Me$C_{31}$ |
| 11 | 16.529 | n-$C_{26}$ | 47 | 25.871 | 11,15-;11,13diMe$C_{31}$ |
| 12 | 17.273 | 4Me$C_{26}$ | 48 | 25.946 | 9,15-;9,13diMe$C_{31}$ |
| 13 | 17.435 | z-$C_{27}$ | 49 | 26.050 | 7,15-;7,13diMe$C_{31}$ |
| 14 | 17.781 | n-$C_{27}$ | 50 | 26.141 | 3Me$C_{31}$ |
| 15 | 18.230 | 13-;11-;9Me$C_{27}$ | 51 | 26.222 | 5,15-;5,13-;5,11diMe$C_{31}$ |
| 16 | 18.480 | 7Me$C_{27}$ | 52 | 26.613 | 3,15-;3,13-;3,11diMe$C_{31}$ |
| 17 | 18.503 | 5Me$C_{27}$ | 53 | 26.969 | 14,18-;14,16diMe$C_{32}$ |
| 18 | 18.900 | 3Me$C_{27}$ | 54 | 27.812 | 13,15diMe$C_{32}$ |
| 19 | 19.022 | 5,11diMe$C_{27}$ | 55 | 27.230 | z-$C_{33}$ |
| 20 | 19.320 | n-$C_{28}$ | 56 | 27.712 | 4,16-;4,14-;4,12diMe$C_{32}$ |
| 21 | 19.546 | 3,13; 3,11 diMe$C_{27}$ | 57 | 27.859 | n-$C_{33}$ |
| 22 | 19.615 | 3,7diMe$C_{27}$ | 58 | 28.410 | 15-;13-;11Me$C_{33}$ |
| 23 | 19.879 | 14-;13-;12-;11-;10-;9-;8Me$C_{28}$ | 59 | 28.644 | 11,15diMe$C_{33}$ |
| 24 | 20.542 | 4Me$C_{28}$ | 60 | 28.838 | 7,13diMe$C_{33}$ |
| 25 | 20.860 | 3Me$C_{28}$ | 61 | 28.984 | 5,15-;5,13diMe$C_{33}$ |
| 26 | 20.938 | z-$C_{29}$ | 62 | 29.421 | 3,15-;3,13diMe$C_{33}$ |
| 27 | 21.304 | n-$C_{29}$ | 63 | 31.036 | 15-;13-;11Me$C_{35}$ |
| 28 | 21.995 | 15-;13-;11-;9Me$C_{29}$ | 64 | 31.709 | 5,15-;5,13diMe$C_{35}$ |
| 29 | 22.086 | 7Me$C_{29}$ | 65 | 31.928 | 3,13diMe$C_{35}$ |

| | | | | | |
|---|---|---|---|---|---|
| 30 | 22.238 | $5MeC_{29}$ | 66 | 33.854 | $15-;13-,11MeC_{37}$ |
| 31 | 22.567 | $9,13diMeC_{29}$ | 67 | 34.323 | $11,XdiMeC_{37}$ |
| 32 | 22.620 | $7,13-;7,11\ diMeC_{29}$ | 68 | 37.773 | $15-;13-;11MeC_{39}$ |
| 33 | 22.772 | $5,15-;5,13-;5,11diMeC_{29}$ | 60 | 38.361 | $11,15-;11,13diMeC_{39}$ |
| 34 | 22.823 | $3MeC_{29}$ | 70 | 38.659 | $7,15-;7,13diMeC_{39}$ |
| 35 | 23.260 | $n\text{-}C_{30}$ | 71 | 39.595 | $3,15-;3,13diMeC_{39}$ |
| 36 | 23.351 | $3,13-;3,11diMeC_{29}$ | 72 | 39.905 | $14MeC_{40}$ |

**Table S3.** Compounds from SIMPER analysis that are important in the separation of the tasks within each treatment with significant p-values.

| **No treatment** | **p** | **Acetone** | **p** |
|---|---|---|---|
| Cell inspector x Builder | | Cell inspector x Builder | |
| $15-;13-;11-;9-;7MeC_{31}$ | 0.043 | $13-;11-;9MeC_{27}$ | 0.0026 |
| $n.C_{31}$ | 0.001 | $15-;14-;13-;12-;11-;10MeC_{30}$ | 0.0009 |
| $5MeC_{27}$ | 0.001 | $15-;13-;11MeC_{39}$ | 0.0001 |
| - | | $5MeC_{27}$ | 0.0001 |
| - | | $5MeC_{29}$ | 0.001 |
| Cell inspector x Forager | | Cell inspector x Forager | |
| $3MeC_{29}$ | 0.001 | $15-;13-;11-;9-;7MeC_{31}$ | 0.0357 |
| $z.C_{29}$ | 0.002 | $3,13-;3.11diMeC_{29}$ | 0.0258 |
| $7,13-;7,11diMeC_{29}$ | 0.001 | $13-;11-;9MeC_{27}$ | 0.0366 |
| - | | $15-;13-;11MeC_{39}$ | 0.0001 |
| - | | $5MeC_{27}$ | 0.0001 |
| Builder x Forager | | Builder x Forager | |
| $3MeC_{29}$ | 0.001 | $n.C_{29}$ | 0.01 |
| $n.C_{27}$ | 0.014 | $5MeC_{29}$ | 0.0033 |
| $3MeC_{27}$ | 0.004 | $5,15-;5,13-;5,11diMeC_{31}$ | 0.0497 |
| $5MeC_{27}$ | 0.001 | - | |
| $n.C_{31}$ | 0.001 | - | |
| $z.C_{29}$ | 0.045 | - | |
| $7,13-;7,11diMeC_{29}$ | 0.001 | - | |
| $15-;14-;13-;12-;11-;10MeC_{30}$ | 0.016 | - | |
| $14-;13-;12-;11-;10-;9-;8MeC_{28}$ | 0.012 | - | |

| **Methoprene** | **p** | **Precocene** | **p** |
|---|---|---|---|
| Cell inspector x Builder | | Cell inspector x Builder | |

| | | | |
|---|---|---|---|
| n.$C_{29}$ | 0.0013 | 11,XdiMeC$_{37}$ | 0.0001 |
| 15-;13-;11-;9-;7MeC$_{31}$ | 0.0001 | 5,15-;5,13-;5,11diMeC$_{29}$ | 0.0001 |
| 3MeC$_{29}$ | 0.0001 | 15-;13-;11MeC$_{39}$ | 0.0001 |
| n.$C_{23}$ | 0.0001 | - | |
| n.$C_{31}$ | 0.0001 | - | |
| 13-;11-;9MeC$_{27}$ | 0.0001 | - | |
| 3,13-;3,11diMeC$_{29}$ | 0.0048 | - | |
| 3,15-;3,13-;3.11diMeC$_{31}$ | 0.0001 | - | |
| 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.0001 | - | |
| 7MeC$_{29}$ | 0.0001 | - | |
| 5,15-;5,13-;5,11diMeC$_{29}$ | 0.0001 | - | |
| 15-;13-;11MeC$_{39}$ | 0.0001 | - | |
| 5MeC$_{27}$ | 0.0001 | - | |
| 5MeC$_{29}$ | 0.0001 | - | |
| 5,15-;5,13-;5,11diMeC$_{31}$ | 0.0021 | - | |
| | | | |
| Cell inspector x Forager | | Cell inspector x Forager | |
| | | | |
| n.$C_{29}$ | 0.0054 | 5,15-;5,13-;5,11diMeC$_{29}$ | 0.0001 |
| 3MeC$_{29}$ | 0.0007 | 11,XdiMeC$_{37}$ | 0.0001 |
| 3MeC$_{27}$ | 0.0001 | 5MeC$_{29}$ | 0.0424 |
| n.$C_{23}$ | 0.0001 | - | |
| n.$C_{27}$ | 0.0153 | - | |
| n.$C_{31}$ | 0.0001 | - | |
| 3,13-;3,11diMeC$_{29}$ | 0.0002 | - | |
| 13-;11-;9MeC$_{27}$ | 0.0002 | - | |
| 3,15-;3,13-;3,11diMeC$_{31}$ | 0.0002 | - | |
| 5,15-;5,13-;5,11diMeC$_{29}$ | 0.0001 | - | |
| 15-;13-;11MeC$_{33}$ | 0.0001 | - | |
| 5MeC$_{27}$ | 0.0001 | - | |
| 7MeC$_{29}$ | 0.0001 | - | |
| 15-;13-;11MeC$_{39}$ | 0.0001 | - | |
| 5MeC$_{29}$ | 0.0145 | - | |
| n.$C_{28}$ | 0.0009 | - | |
| | | | |
| Builder x Forager | | Builder x Forager | |
| | | | |
| n.$C_{27}$ | 0.0222 | 15-;13-;11MeC$_{39}$ | 0.0001 |
| - | | 14-;13-;12-;11-;10-;9-;8MeC$_{28}$ | 0.0354 |

**Table S4.** Results from comparing the chemical profile of workers within each task between the treatments. * Significant p-values.

| **Cell inspector** | NTW | STW | MTW |
|---|---|---|---|
| NTW | | $p < 0.01$* | $p < 0.01$* |
| MTW | $p < 0.01$* | $p < 0.01$* | |
| PTW | $p < 0.01$* | $p < 0.01$* | $p < 0.01$* |
| **Builder** | NTW | STW | MTW |
| NTW | | $p < 0.01$* | $p < 0.01$* |
| MTW | $p < 0.01$* | $p = 0.22$ | |
| PTW | $p = 0.01$* | $p = 0.20$ | $p = 0.35$ |
| **Forager** | NTW | STW | MTW |
| NTW | | $p = 0.01$* | $p = 0.02$* |
| MTW | $p = 0.01$* | $p = 0.02$* | |
| PTW | $p = 0.02$* | $p = 0.15$ | $p = 0.01$* |

**Table S5.** Compounds from SIMPER analysis that are important in the separation of the treatments within each task with significant p-values.

| **Cell inspector** | **p** | **Builder** | **p** | **Forager** | **p** |
|---|---|---|---|---|---|
| NTW x MTW | | NTW x MTW | | NTW x MTW | |
| $n.C_{29}$ | 0.002 | 15-;13-;11-;9$MeC_{29}$ | 0.001 | 3,15-;3,13-;3,11di$MeC_{31}$ | 0.004 |
| $3MeC_{29}$ | 0.001 | $n.C_{31}$ | 0.001 | 7,13-;7,11di$MeC_{29}$ | 0.001 |
| $n.C_{31}$ | 0.001 | $5MeC_{27}$ | 0.001 | $z\text{-}C_{29}$ | 0.039 |
| $n.C_{23}$ | 0.001 | - | | - | |
| 13-;11-;$9MeC_{27}$ | 0.004 | - | | - | |
| $3MeC_{27}$ | 0.024 | - | | - | |
| 15-;14-;13-;12-;11-;$10MeC_{30}$ | 0.002 | - | | - | |
| 15-;13-;$11MeC_{33}$ | 0.001 | - | | - | |
| $7MeC_{29}$ | 0.001 | - | | - | |
| 5,15-;5,13-;5,11di$MeC_{29}$ | 0.001 | - | | - | |
| 15-;13-;$11MeC_{39}$ | 0.001 | - | | - | |
| $5MeC_{27}$ | 0.001 | - | | - | |
| $5MeC_{29}$ | 0.001 | - | | - | |
| NTW x PTW | | NTW x PTW | | NTW x PTW | |
| 15-;13-;11-;$9MeC_{29}$ | 0.004 | $n.C_{31}$ | 0.001 | 15-;13-;$11MeC_{39}$ | 0.001 |
| 11,Xdi$MeC_{37}$ | 0.001 | $5MeC_{27}$ | 0.001 | 7,13-;7,11di$MeC_{29}$ | 0.001 |
| 15-;13-;$11MeC_{39}$ | 0.001 | $n.C_{28}$ | 0.018 | - | |
| 5,15-;5,13-;5,11di$MeC_{29}$ | 0.001 | - | | - | |
| NTW x STW | | NTW x STW | | NTW x STW | |
| 15-;13-;$11MeC_{39}$ | 0.001 | $n.C_{31}$ | 0.036 | 15-;13-;11-;$9MeC_{29}$ | 0.042 |
| $5MeC_{27}$ | 0.001 | $5MeC_{27}$ | 0.001 | $z.C_{29}$ | 0.003 |

| | | | | | |
|---|---|---|---|---|---|
| - | | 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.002 | 7,13-;7,11diMeC$_{29}$ | 0.001 |
| - | | 5MeC$_{29}$ | 0.001 | n-C$_{31}$ | 0.039 |

| MTW x PTW | | MTW x PTW | | MTW x PTW | |
|---|---|---|---|---|---|
| 15-;13-;11-;9-;7MeC$_{31}$ | 0.003 | n.C$_{29}$ | 0.026 | 3,13-;3,11diMeC$_{29}$ | 0.041 |
| 3MeC$_{29}$ | 0.014 | 3,13-;3,11diMeC$_{29}$ | 0.044 | 3MeC$_{27}$ | 0.026 |
| n.C$_{23}$ | 0.001 | - | | 3,15-;3,13-;3,11diMeC$_{31}$ | 0.009 |
| n.C$_{31}$ | 0.001 | - | | 15-;13-;11MeC$_{39}$ | 0.001 |
| 13-;11-;9MeC$_{27}$ | 0.008 | - | | 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.040 |
| 3,15-;3,13-,3,11diMeC$_{31}$ | 0.006 | - | | - | |
| 3MeC$_{27}$ | 0.001 | - | | - | |
| 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.003 | - | | - | |
| 11,XdiMeC$_{37}$ | 0.001 | - | | - | |
| 7MeC$_{29}$ | 0.001 | - | | - | |
| 15-;13-;11MeC$_{33}$ | 0.038 | - | | - | |
| 5MeC$_{27}$ | 0.001 | - | | - | |
| 5MeC$_{29}$ | 0.001 | - | | - | |

| MTW x STW | | MTW x STW | | MTW x STW | |
|---|---|---|---|---|---|
| 3MeC$_{29}$ | 0.004 | 3MeC$_{29}$ | 0.009 | 3,13-;3,11diMeC$_{29}$ | 0.007 |
| n.C$_{23}$ | 0.001 | 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.003 | 3MeC$_{27}$ | 0.010 |
| n.C$_{31}$ | 0.001 | 5MeC$_{29}$ | 0.011 | n.C$_{28}$ | 0.049 |
| 3,13-;3,11diMeC$_{29}$ | 0.005 | - | | 5,15-;5,13-;5,11diMeC$_{31}$ | 0.033 |
| 0.0333,15-;3,13-;3.11diMeC$_{31}$ | 0.001 | - | | - | |
| 13-;11-;9MeC$_{27}$ | 0.006 | - | | - | |
| 3MeC$_{27}$ | 0.001 | - | | - | |
| 7MeC$_{29}$ | 0.001 | - | | - | |
| 5,15-;5.13-;5.11diMeC$_{29}$ | 0.001 | - | | - | |
| 15-;13-;11MeC$_{33}$ | 0.003 | - | | - | |
| 5MeC$_{29}$ | 0.007 | - | | - | |
| n.C$_{28}$ | 0.005 | - | | - | |
| 5,15-;5,13-;5,11diMeC$_{31}$ | 0.001 | - | | - | |

| PTW x STW | | PTW x STW | | PTW x STW | |
|---|---|---|---|---|---|
| 11,XdiMeC$_{37}$ | 0.001 | 13-;11-;9MeC$_{27}$ | 0.023 | 15-;13-;11MeC$_{39}$ | 0.001 |
| 5,15-;5,13-;5,11diMeC$_{29}$ | 0.001 | 15-;14-;13-;12-;11-;10MeC$_{30}$ | 0.006 | - | |
| 5MeC$_{27}$ | 0.001 | 14-;13-;12-;11-;10-;9-;8MeC$_{28}$ | 0.004 | - | |
| | | 15-;13-;11MeC$_{33}$ | 0.043 | - | |

**Table S6.** Comparison of ovaries types between the treatments in each task. * Significant p-values.

| **Cell inspector** | | |
|---|---|---|
| | z | p |
| NTW x STW | -0.795 | 0.4282 |
| NTW x MTW | -2.694 | 0.0424 |
| NTW x PTW | 1.986 | 0.0941 |
| STW x MTW | 2.288 | 0.0664 |
| STW x PTW | 1.464 | 0.2149 |
| MTW x PTW | -0.792 | 0.4282 |
| **Builder** | | |
| | z | p |
| NTW x STW | 0.228 | 0.8196 |
| NTW x MTW | -3.189 | <0.01* |
| NTW x PTW | 1.546 | 0.1752 |
| STW x MTW | 2.685 | 0.0217* |
| STW x PTW | 1.454 | 0.1752 |
| MTW x PTW | -1.992 | 0.0927 |
| **Forager** | | |
| | z | p |
| NTW x STW | -0.378 | 0.8465 |
| NTW x MTW | -2.889 | <0.01* |
| NTW x PTW | -0.164 | 0.8701 |
| STW x MTW | 2.831 | <0.01 |
| STW x PTW | -0.558 | 0.8465 |
| MTW x PTW | -3.122 | <0.01* |