Retained metabolic activity in honey bee collected pollen has implications for pollen digestion and effects on honey bee health

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Abstract – The mechanisms by which pollen is digested by honey bees are incompletely understood. Potential methods are thought to include pseudogermination, mechanical disruption, enzymatic breakdown, or osmotic shock. Understanding the role of pseudogermination in this process has been hampered by a lack of tools demonstrating retention of metabolic activity in pollen collected by honey bees. Here, we show that pollen collected by honey bees produces reactive oxygen species (ROS) at robust levels upon germination, suggesting that ROS is a suitable marker of this process in pollen. ROS can be readily found in the digestive tract of honey bees and is localized to pollen grains within the lumen. Finally, manipulating pollen levels in the midgut can change ROS levels in the digestive tract. These data provide evidence of retained metabolic activity in bee-collected pollen that lends support to pseudogermination as a mechanism for pollen digestion in honey bees, and points to novel approaches for better understanding of pollen digestion in this species and beyond.

ecology / honey bee / pollen / pollinator / digestion

1. INTRODUCTION

Simply defined, pollination is the transfer of a pollen grain from the anther of one flower to the stigma of the same or another flower. Many plants rely on pollinators to facilitate this process, providing nutrients to pollinators in exchange for the reproduction-enabling service of pollination (reviewed in (Waser and Ollerton 2006)). The western honey bee, *Apis mellifera*, is a vitally important participant in this process in both

agricultural and ecological settings (reviewed in (Potts et al. 2016)).

For honey bees, pollen represents an important nutrient source (reviewed in (Wright et al. 2018)), but the way it is digested in this species is incompletely understood. Pollen grains contain 1–2 sperm cells and one vegetative cell which are protected by an inner layer (intine) and a tough outer layer (exine) and are dehydrated and metabolically inactive for dispersal (reviewed in {Borg:2011ee}). In other palynivorous species, pollen digestion is thought to involve one or several of the following mechanisms: induction of germination/pseudogermination, mechanical disruption of the pollen wall, enzymatic breakdown, and osmotic shock (Roulston and Cane 2000). Current evidence supports two of these four pathways for pollen digestion in honey bees: pseudogermination and osmotic shock (Peng and Dobson 1997).

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Osmotic shock occurs when the osmotic pressure differential between the crop and ventriculus causes the pollen grain to rupture when traversing between these compartments. In vitro evidence suggests this process may occur in honey bee digestion (Kroon et al. 1974). However, in situ studies demonstrate a slow loss of pollen contents in the midgut, in line with an enzymatic digestion or pseudogermination process as opposed to osmotic shock (Klungness and Peng 1983; Klungness and Peng 1984a; Klungness and Peng 1984b; Peng et al. 1985; Peng et al. 1986; Human and Nicolson 2003). Once the contents of the pollen grain are unprotected, digestion of macromolecules proceeds, via enzymes that are likely produced by both honey bee epithelial cells and resident microbiota (Wright et al. 2018).

Pseudogermination occurs when the pollen grain initiates a germination sequence, but is interrupted prior to full pollen tube formation. A significant body of work shows that pollen collected and stored by honey bees has reduced capacity to pollinate female flowers, in part due to loss of germination potential (Singh and Boynton 1949; Griggs and Vansell 1950; Griggs et al. 1953; Johansen 1956; Kraai 1962; Free and Durrant 1966; Klungness et al. 1983; Mesquida and Renard 1989; Vaissiere et al. 1996). Post collection, further processing and storage in the comb may change germination potential as well. A pseudogermination mechanism of digestion would require that some aspects of germination, such as metabolic activity, remain intact despite the loss of germination potential. While older studies suggest that progressive microbiological and biochemical changes render honey bee stored and consumed pollen metabolically inactive (Gilliam 1997), recent studies suggest that the biochemical and microbiological characteristics of pollen do not change substantially upon collection or typical storage as bee bread (Human and Nicolson 2006; Nicolson and Human 2013; Anderson et al. 2014).

The aforementioned studies predominantly focused on pollen germination as defined by pollen tube formation or fertilization potential, both of which concern late or final events in the germination process. By contrast, pseudogermination is likely to be an interrupted process. Recent

advances in our understanding of the cellular and molecular steps involved in the early stages of germination may provide novel tools for understanding the process of pseudogermination. For example, germination results in the production of an early “wave” of reactive oxygen species (ROS) that provides key signaling cues for pollen function (reviewed in (McInnis et al. 2006)). We therefore propose that a ROS burst in honey bee-collected pollen could provide additional evidence of retained metabolic activity and potential pseudogermination during honey bee pollen digestion. Here, we show that pollen collected by honey bees from multiple plant species produces ROS at robust levels upon germination, both in vitro and in the lumen of the digestive tract. Further, we show that manipulating pollen levels in the midgut can change ROS levels in the digestive tract. These data provide evidence of retained metabolic activity in bee-collected pollen that lends support to pseudogermination during pollen digestion in honey bees, and points to novel approaches for better understanding of pollen digestion in this species and beyond.

2. MATERIALS AND METHODS

2.1. Honey bee and pollen collection

Honey bees were collected from outbred colonies in New York, NY, during the months of April–October in 2014 and 2015. Colonies were visually inspected for symptoms of common diseases, and only visibly healthy bees were collected. For feeding experiments, bees returning to the hive without pollen loads were collected. Pollen used for in vitro germination and feeding experiments was collected from the *corbiculae* of returning foragers from the colonies above (Figure 1a).

2.2. Species identification of honey bee-collected pollen

To determine the species identity of pollen collected by honey bees, the rRNA *internal transcribed spacer 2* (*ITS2*) region was sequenced for one of two paired pollen loads on the *corbiculae* of a single forager. The other paired pollen load

was used immediately in the Amplex Red assay (see below) to assess ROS production. Honey bees are well known to display flower constancy, preferring to visit flowers of the same species in a foraging trip (reviewed in (Brodschneider et al. 2018)). In fact, it has been found that less than 3% of pollen loads are mixed in pollen composition (Betts 1935). To obtain ITS2 sequence from pollen, RNA was extracted using Trizol Reagent (Invitrogen, San Diego, CA) per manufacturer's instruction after manually crushing the pollen with a disposable pestle. cDNA was synthesized using the iScript cDNA Synthesis Kit (Biorad, Hercules, CA) and 1–5 μ l of cDNA was then used as a template for PCR using universal plant ITS2 primer sequences used (ITS2F: 5'- ATGCGATA CTTGGTGTGAAT-3' and ITS3R: 5'- GACGCTTCTCCAGACTACAAT-3'). PCR was performed with using the GoTaq polymerase and buffer (Promega, Madison, WI); cycling conditions have been described elsewhere (Chen et al. 2010). After visualizing a fraction of the PCR products on a gel, the remainder was purified using a QIAGEN PCR Purification Kit (Genewiz, NJ) and sequenced using Sanger sequencing sequences were annotated and the genus (and highest ranked species) of the plant of origin was identified using the ITS2 Database (Koetschan et al. 2009).

2.3. Pollen counts

Pollen grains were diluted to 10 μ g/ μ l in PBS and boli were crushed in 600 μ l for the Amplex Red assay above. For pollen from midgut contents, an aliquot of 50 μ l was stained with Safranin O for pollen counting (Jones 2012). Pollen counts for a known area of the slide were made using a Nikon SMZ1500 stereo zoom microscope and multiplied by the whole slide area. The total number of pollen grains was calculated using total volume of the sample and total area of the slide.

2.4. Visualization of ROS in pollen and food boli

Pollen was collected from the *corbiculae* of returning foragers and analyzed on the day of acquisition. Pollen grains were hydrated in PBS

Figure 1. Reactive oxygen species (ROS) production in honey bee-collected pollen. **a** Typical pollen load on the *corbiculae* of foragers returning to the hive. **b** Bee-collected pollen grains assayed for ROS presence using CM-H₂DCF. Fluorescence indicates ROS. From top: (i) Control with no CM-H₂DCF dye, (ii) CM-H₂DCF dye without inhibitors, (iii) CM-H₂DCF dye with inhibitor N-acetyl cysteine (NAC), (iv) CM-H₂DCF dye with inhibitor ascorbic acid (AA). Left panels are brightfield images, right panels are phase-contrast images ($\times$ 100 magnification, scale bar = 0.1 mm).

and diluted to 10 μ g/ μ l. To examine ROS within the midgut lumen, the food bolus was removed from the midgut by removing the peritrophic matrix and its contents as before (Masood et al. 2016).

For ROS detection in both pollen and midgut food bolus samples, samples were incubated at room temperature for 20 min with 0.1 mM 5-(and 6) - c h l o r o m e t h y l - 2', 7' - dichlorodihydrofluorescein diacetate, acetyl ester (CM-H₂DCFDA; Molecular Probes, Eugene, Ore) in reaction buffer as per manufacturer's instructions. For food bolus samples, fluorescence was also recorded prior to adding CM-H₂DCFDA to assess the presence of auto-fluorescence. ROS-generated fluorescence was visualized by a Nikon SMZ1500 stereo zoom microscope with brightfield/darkfield base, fluorescence unit, and Nikon Digital Sight DS-Fi1 high-resolution digital camera, with controller and PC.

2.5. Measurement of hydrogen peroxide

Hydrogen peroxide in pollen was quantified as a measure of ROS production using the Amplex Red assay. For measurements of H₂O₂ in fresh pollen and bee bread, pollen grains were diluted to 10 μ g/ μ l in PBS and 1 mg was used per replicate of each assay condition. The assay was performed using the Amplex Red Assay (Invitrogen) as per the manufacturer's instructions with or without 200 mM N-acetyl cysteine (NAC) or 50 mM ascorbic acid (AA) in a final volume of 300 μ l. For heat inhibition (HI), $\frac{1}{2}$ of the pollen was heated at 80 °C for 1 h before starting the assay. For pre-germination, $\frac{1}{2}$ of pollen was allowed to germinate for 2 h, washed, and resuspended

before starting the assay. After incubation at 37 °C for 30 min, the H₂O₂ level was measured spectrophotometrically at 560 nm (A560nm) using a *GloMax-Multi Jr* Single-Tube Multimode Reader (Promega). For each pollen sample, a negative control, in which only the Amplex red reagent was omitted, was performed.

For measurements of H₂O₂ in midgut food boli, each bolus was crushed in 600 µl solution, and 100 µl was used in each replicate of each assay condition. In the case of inhibitors, multiple boluses were mixed (again with a final concentration of 600 µl per bolus). The assay was again performed using the Amplex Red Assay (Invitrogen) as per the manufacturer's instructions with or without 200 mM N-acetyl cysteine (NAC) or 50 mM ascorbic acid (AA) in a final volume of 300 µl. In addition, control samples without pollen or bolus, which were spiked with 0 or 10 µM of H₂O₂ were performed for each experiment. Low signal was sometimes observed in food boluses without the Amplex Red reagent so this background was subtracted to obtain bolus ROS levels for individual bees.

2.6. Measurement of superoxide anion production

Superoxide anion concentration in collected pollen as a measure of ROS production was assessed using a nitroblue tetrazolium assay. Pollen grains were diluted to 10 µg/ul in PBS and 1 mg was used per assay condition. The assay was performed using 2 mM nitroblue tetrazolium (NBT) in a final volume of 300 µl. Mixtures were then incubated for 15 min at 37 °C. NBT was completely removed by repeated washing steps in PBS followed by a final methanol wash. The formazan precipitate was dissolved in 2 M KOH followed by dimethyl sulfoxide (DMSO) (Rook et al. 1985). Absorbance was determined at 530 nm (A530nm) on a spectrophotometer (DU 530; Beckman Instruments, Fullerton, CA).

2.7. Feeding treatments

For all caged experiments, approximately 15 honey bees without pollen (unless otherwise noted) were selected from the landing board of

colonies as above and kept in 177.4 mL (6 oz.) square-bottomed *Drosophila* Stock Bottles (VWR) plugged with modified foam tube plugs (Jaece Industries). Bees were maintained in incubators at 35 °C in the presence of PseudoQueen (Contech, Victoria, British Columbia, Canada) as a source of Queen Mandibular Phermone (QMP), which was used as per manufacturer's instructions. Bees were fed via a modified 1.5-ml screw-cap tube. Bees were fed a diet of sucrose syrup alone for 24 h, starved for 1 h to encourage feeding, and then fed 30% sucrose with or without monofloral or polyfloral pollen for 1 h (as described in the results) provided at a concentration of 50 mg/ml. No differences in consumption were observed between groups and the mortality in these short-term experiments was essentially non-existent.

2.8. Statistical analysis

For all spectrophotometry data, mean values were calculated for each group. For in vitro assays, normality was assumed due to the small sample size. For in vivo assays, normality was assessed using Shapiro–Wilk tests or Kolmogorov–Smirnov as noted in the statistics table. Differences between two groups were compared using unpaired *t* tests with Welch's correction when values fit normal distributions or Mann–Whitney *U* nonparametric tests when they did not fit normal distributions. Differences between more than two groups were compared using ANOVA or Kruskal–Wallis test with post-hoc Tukey's multiple comparison test. All statistical data can be found in Supplemental Document 1.

3. RESULTS

3.1. Pollen ROS levels vary across different plant species

We identified six plant species representing five genera in honey bee-collected pollen from different sampling periods (Table I). Using the Amplex Red assay, we observed variable levels of ROS production by pollen from different species in our assay, and some pollen types did not appear to produce ROS (Table I and Suppl.

Table 1. Species identification and ROS production activity of honey bee–collected pollen

Species	Date collected	ROS production ($\mu\text{M H}_2\text{O}_2$)
<i>Plantago rugelii</i>	07/25/14	0.23 (± 0.094) [†]
<i>Trifolium repens</i>	07/25/14	5.47 (± 0.123)**
<i>Trifolium repens</i>	08/03/14	4.98 (± 0.025)**
<i>Trifolium pretense</i>	08/03/14	1.04 (± 0.493) [†]
<i>Styphnolobium japonicum</i>	08/04/14	4.29 (± 0.068)*
<i>Ageratina altissima</i>	10/03/13	0.73 (± 0.39) [†]
<i>Ageratina altissima</i>	10/03/13	2.05 (± 0.46)*
<i>Symphytotrichum spathulatum</i>	10/03/13	0.93 (± 0.084)**

Mean with Amplex with mean of no Amplex control subtracted ($\pm$ SEM)

[†] Not significantly different than no Amplex control

* $p < 0.05$ relative to no Amplex control

** $p < 0.01$ relative to no Amplex control

Figure 1). However, at each time point, we found at least one plant species with metabolically active pollen. We also found that pollen samples from different genera produce variable levels of ROS upon germination (Table 1).

3.2. Honey bee–collected pollen produces robust ROS levels in vitro

We found robust signal of ROS in pollen grains collected from *corbiculae* using the CM-H2DCFDA indicator reagent (Figure 1b). We were able to eliminate the signal through the addition of antioxidants N-acetyl cysteine (NAC) or ascorbic acid, (AA) demonstrating that the signal represents ROS.

We also found robust ROS signal in pollen grains using the Amplex Red Assay (Figure 2a). Again, the signal could be eliminated through the addition of antioxidants, demonstrating the signal is specific for ROS (Figure 2b). We also found that the signal could be eliminated through heat inhibition (HI), demonstrating the signal is dependent on active enzymes in living pollen (Figure 2c). In addition, pre-germination for 2 h abolished the signal, demonstrating that ROS is transiently produced as a result of pollen activation (Figure 2d). We also used the nitroblue tetrazolium (NBT) assay, which detects the presence of superoxide anion. After

NBT treatment, we found formation of formazan deposits, indicating the presence of ROS. These three assays demonstrate that pollen collected from honey bee *corbiculae* possesses the ability to produce ROS upon rehydration (Figure 2e).

3.3. Pollen in honey bee midgut contents produces ROS

We found a robust signal of ROS in the food bolus of individual bees using the indicator CM-H2DCFDA. The signal was often present in structures that appeared to be pollen grains (Figure 3a). Using the Amplex Red assay, we found variable signal in food boluses of individual bees (Figure 3b). When boluses were pooled, signal could be eliminated through the addition of NAC or AA, demonstrating the signal is specific for ROS (Figure 3c).

The above sampled bees were foragers, which are thought to consume relatively little pollen (Crailsheim et al. 1992; Crailsheim et al. 1993; Hrassnigg and Crailsheim 1998) in part because of their minimal ability to digest it (Moritz and Crailsheim 1987). However, we consistently found some pollen in the midguts of returning foragers, perhaps suggesting sampling of pollen or accidental ingestion at the flower. If so, foragers that return to

Figure 2. ROS production in honey bee-collected pollen is sensitive to anti-oxidants and heat inactivation. **a–d** Results of Amplex Red Assay. H₂O₂ standards are presented on the far right; + and – represent with or without dye. **a** H₂O₂ production by bee-collected pollen grains from four different corbicular loads (numbered 1–4 on x-axis) harvested on the same day. **b** H₂O₂ production by bee-collected pollen grains with or without N-acetyl cysteine (NAC) or ascorbic acid (AA). **c** H₂O₂ production by bee-collected pollen grains with or without heat inactivation (HI). **d** H₂O₂ production by bee-collected pollen grains with or without pre-germination (PG). **e** Superoxide anion production by pollen grains demonstrated by NBT assay. Data is represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, $a \neq b \neq c$ $p < 0.05$

the nest without pollen in their *corbiculae* might represent nectar foragers and therefore

be expected to have ingested little pollen. When comparing the gut contents of returning

Figure 3. ROS production in honey bee food boluses. **a** Food bolus contents assayed for ROS presence using CM-H₂DCF. Fluorescence indicates ROS. From top: (i) bolus with no CM-H₂DCF dye, (ii) bolus with CM-H₂DCF dye (iii) bolus with CM-H₂DCF dye at higher magnification. Left panels are brightfield images, right panels are phase-contrast images of each bolus (scale bar = 0.1 mm for i/ii and 0.5 mm for iii). **b** H₂O₂ production by food boluses from forager honey bees detected by the Amplex Red assay. Plot shows 1st and 3rd interquartile range with lines denoting medians. Whiskers encompass 95% of the individuals. Outliers are denoted with circles. **c** H₂O₂ production by food boluses from forager honey bees with or without N-acetyl cysteine (NAC) or ascorbic acid (AA) detected by the Amplex Red assay; + and – represent with or without dye. Data is represented as mean ± SEM. **p* < 0.05, ***p* < 0.01, *a* ≠ *b* *p* < 0.05.

foragers that were carrying pollen to those that were not, we did in fact observe that bees with pollen in their *corbiculae* had more pollen in their midgut contents than returning bees not carrying pollen and more ROS (Figure 4a, b).

3.4. Honey bee pollen consumption results in ROS production

To demonstrate that preventing ingestion of pollen lowers levels of ROS in the gut, we collected returning foragers with pollen loads and measured the midgut ROS levels in one group immediately. We fed the remaining group a pollen-free diet for 24 h, and then measured ROS levels. We found that ROS levels and pollen counts both declined in bees fed a pollen free diet for 24 h, compared with foragers that were sampled immediately upon return (Figure 5a, b). please check Figure 5.

To demonstrate that pollen consumption leads to increased levels of ROS in honey bee midguts, we fed bees sucrose syrup for 24 h, starved them for 1 h, then fed them either sucrose syrup alone or sucrose syrup supplemented with 50 mg/ml fresh pollen for 1 h. We observed increased ROS levels (Figure 5c) and pollen counts (Figure 5d) in bees fed sucrose syrup with pollen compared with

those receiving sucrose syrup alone for the same time period.

4. DISCUSSION

We observe that honey bee–collected pollen from multiple plant species can produce ROS, both in vitro and in the honey bee gut. We also observe that ROS is extinguished by antioxidants and that its production can be reduced by heat inactivation or by pre-germination of pollen samples, providing strong evidence that honey bee–collected pollen retains some metabolic activity that is initiated upon exposure to fluid, potentially representing partial or pseudo germination.

Most studies of germination potential post-collection have focused on pollen tube formation or fertilization potential (Roulston and Cane 2000). These are late events in the germination process and loss of these functions is not necessarily associated with loss of the ability to initiate the germination process. By focusing on metabolic activity associated with early germination processes, our results support a mechanism in which pollen grains collected by bees can initiate the germination process. In fact, retained metabolic activity would be essential for initiation of pseudogermination even as an interrupted process. Our results are consistent with earlier studies

Figure 4. Foragers returning with pollen have higher pollen counts and higher ROS levels in the food bolus. **a** H_2O_2 production as detected by the Amplex Red assay and **b** pollen counts as assessed by Safranin O staining in food boluses from forager honey bees with (+, $n = 11$) or without (–, $n = 11$) corbicular loads containing pollen. Data is represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, $\neq$ b $p < 0.05$.

Figure 5. Manipulation of pollen levels in the midgut changes ROS levels in the food bolus. **a** H_2O_2 production as detected by the Amplex Red assay (“0”, $n = 7$) and after (“24”, $n = 9$) and **b** pollen counts (“0”, $n = 5$) and after (“24”, $n = 5$) as assessed by Safranin O staining in food boluses from forager honey bees before being maintained on a pollen-free diet for 24 h. **c** H_2O_2 production as detected by the Amplex Red assay and **d** pollen counts as assessed by Safranin O staining in food boluses from forager bees immediately after collection ($n = 7$) or after being fed pollen for 1 h ($n = 7$). Data is represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, a'b $p < 0.05$

that found that bee-collected pollen showed a significant increase in O_2 uptake relative to ungerminated pollen (Keularts and Linskens 1968; Verhoef and Hoekstra 2012), supporting retained metabolic activity despite absence of pollen tube formation. Future studies of early germination processes (e.g., through the use of Ca^{2+} flux ((Steinhorst and Kudla 2013), structural cellular changes (Vogler et al. 2015), and transcriptomics and proteomics (Ambrosino et al. 2016)) may provide additional understanding of pollen digestion in this species. By investigating these early-stage cellular components and processes in the honey bee gut, future studies may yield additional

information that cannot be captured in studies of pollen tube formation or fertilization potential alone.

We observed significant differences between the ROS production by pollen representing different plant species and even differences in the levels of ROS in pollen from the same species. While it is interesting to speculate about the significance of the observed differences between species, there are a number of caveats to such comparisons in this assay. First, pollen from different species is known to possess quite different requirements for initiation of germination, including media components and time after hydration. Therefore, it is

quite possible that our PBS-based germination media is not appropriate for germination of certain pollen types. Second, other pollen indices, such as protein content and viability, are known to vary extensively between members of the same species, based on genotypic and environmental influences on plant health (Marshall et al. 2010; Yeaman et al. 2014). For example, pollen is known to lose germination potential under a number of conditions in a species-specific manner. A variable known to affect germination is the time since production in the anther. This ability is dependent on whether the species produces pollen of the orthodox (low-metabolism) or recalcitrant (high-metabolism) type (Hashida et al. 2013). Flowering plants generally produce pollen in a narrow window of time and we are unable to accurately determine the age of the pollen used in the assay. In addition, the pollen type of each species has not always been described, making it difficult to predict pollen longevity and ROS activity.

In addition to our *in vitro* assays, we also find that ROS can be readily found in the lumen of the digestive tract of honey bees and appears localized to pollen grains, suggesting that initiation of metabolic activity occurs to some extent during the pollen digestion process. Furthermore, manipulating pollen levels in the midgut can change ROS levels in the digestive tract; an increase in gut pollen through feeding leads to an associated increase in ROS in the lumen, while a decrease in pollen feeding by feeding sucrose solution alone leads to a decrease in ROS. It is important to note that changes in ROS levels in the midgut could also be due to alterations in metabolism of the epithelial cells in response to the presence or absence of pollen in the diet. In addition, increased ROS levels in the midgut after feeding could also be due to ROS production by the epithelial cells in response to oral exposure to microbes, as has been shown in other invertebrate midguts (reviewed in (Lee et al. 2017)). A similar inducible ROS system has been proposed in honey bees (Kawahara et al. 2007; Dussaubat et al. 2012). However, in a separate series of experiments, we did not observe any evidence of increased ROS production after feeding bees

either the pathogenic bacterium *Serratia marcescens* or the proposed cellular agent of inducible ROS production (the DUOX ligand uracil) (data not shown). In addition, we observed that ROS in the gut lumen was localized to pollen grains, which seems an unlikely finding if changes in ROS levels in the midgut were due to ingestion induced ROS production by the epithelial cells.

In addition to providing potential insight into pollen digestion, the observed increase in ROS levels after pollen consumption may have other implications for honey bee health. Previous work has shown that ROS produced by pollen from wind-pollinated species may damage the barrier epithelial tissue of the airway in humans in the context of allergic inflammation (Boldogh et al. 2005; Bacsı et al. 2005; Wang et al. 2009; Speranza and Scoccianti 2012). Pollen-derived ROS could have significant effects on the biology of the barrier epithelia in the honey bee as well. First, ROS could cause damage to epithelial cells and impact barrier epithelial function, as has been reported after chemical induced ROS exposure in fruit fly digestive tract (Chatterjee and Ip 2009; Buchon et al. 2009). Second, pollen-derived ROS could negatively impact the microbiota of the digestive tract by exposing the resident bacteria to this antimicrobial compound (Lee et al. 2013).

Significant further study is needed to fully understand the impacts of the pollen associated ROS on honey bees in the context of the colony. Pollen consumption by worker honey bees is quite variable and depends on developmental stage and age. Pollen represents less than 5% of the total protein consumed during larval development (Babendreier et al. 2004), in contrast to the large quantities of pollen that young workers consume after they emerge as adults from their cells (Hagedorn and Moeller 1967; Dietz 1969). Thus, adult workers are the group that is most likely to consume pollen and be affected by ROS production. However, honey bee workers also exhibit an age-based societal structure, in which a major behavioral transition occurs with a switch from nursing inside the nest to foraging outside (Winston 1987). One hallmark of this transition is reduced protein consumption, manifested as a reduction in both the amount of pollen a worker

ingests (Crailsheim et al. 1992; Crailsheim et al. 1993; Hrassnigg and Crailsheim 1998) and her ability to digest pollen due to reduced production of digestive enzymes (Moritz and Crailsheim 1987). Thus, the effect of ROS production (and potentially pseudogermination) during pollen digestion will likely differ between workers of different classes within colonies, and may impact foraging and feeding decisions on both individual and colony levels.

Here, we provide evidence of preserved metabolic activity in pollen collected and consumed by honey bees. Such retained metabolic activity would be essential for initiation of pseudogermination events and thus these data provide additional support for a mechanism of pollen digestion that includes pseudogermination in honey bees, and points to novel approaches for better understanding of pollen digestion in this species and other palynivorous insects.

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AUTHORS' CONTRIBUTIONS

MM, SRP, and JWS conceived and designed the experiments. MM, SRP, TRA, and JWS performed experiments and analyzed the data. All authors contributed to the drafting and revision of the article.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

Le maintien de l'activité métabolique du pollen a des répercussions sur la digestion du pollen et des effets sur la santé des abeilles.

écologie / abeilles mellifères / pollen / pollinisateur / digestion.

Reste metabolischer Aktivität in Honigbienenpollen sind von Bedeutung für die Pollenverdauung und die Gesundheit von Honigbienen.

Ökologie / Honigbiene / Pollen / Bestäuber / Verdauung.

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