



Division of labor is associated with age-independent changes in ovarian activity in *Pogonomyrmex californicus* harvester ants

Adam G. Dolezal^{a,*}, Joshua Johnson^b, Bert Hölldobler^{a,c}, Gro V. Amdam^{a,d}

^a School of Life Sciences, Arizona State University, Tempe, AZ, USA

^b Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University, New Haven, CT, USA

^c Biocenter, Behavioral Physiology and Sociobiology, University of Würzburg, Germany

^d Department of Chemistry, Biotechnology and Food Science, University of Life Sciences, Aas, Norway

ARTICLE INFO

Article history:

Received 9 November 2012

Received in revised form 18 February 2013

Accepted 19 February 2013

Available online 6 March 2013

Keywords:

Ovary

Behavioral physiology

Division of labor

Formicidae

Social evolution

Reproductive ground plan

ABSTRACT

An age-independent division of labor can develop in both the reproductive (queen) and non-reproductive (worker) castes of *Pogonomyrmex californicus* harvester ants, and individuals develop biases for in-nest activities or external foraging. Additionally, ant ovaries normally atrophy in foragers compared to nest-biased workers (nurses). However, it is not clear whether these ovarian changes are due to changes in behavior or age, since foragers are typically older individuals. Here, we clarify this relationship in *P. californicus* queens and workers by comparing ovarian activity in same-aged ants that exhibit divergent behavioral biases. We found that foraging individuals had significantly reduced ovarian activity compared to their nest-biased counterparts, thereby linking changes in the ants' reproductive system to social task performance rather than to age. The general finding that ovarian physiology is associated with social insect behaviors is consistent with the hypothesis that reproductive physiology may have played an important role in the evolution of social insect behavior.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

In most eusocial insect species, the success of the colony hinges on the completion of a variety of tasks, including food acquisition, brood care, and nest maintenance. These tasks are completed by a cooperative division of labor within the predominantly sterile worker caste. This division in task performance is age-based, with younger workers performing mostly in-nest tasks, like brood care, and older workers foraging for food or defending the nest (Hölldobler and Wilson, 2009). Since the ability of workers to switch between different tasks is an important component of the widespread ecological success of eusocial insects (Wilson, 1971), investigating the underlying physiology behind behavior helps us understand both how insect societies are regulated and how they evolved.

The proximate basis of temporal polyethism has been most heavily investigated in the honey bee, *Apis mellifera* (Ben-Shahar et al., 2002; Amdam et al., 2004, 2006; Rueppell et al., 2004; Hunt et al., 2007; Wang et al., 2010). Despite the fact that honey bee workers do not normally reproduce, regulators normally associated with reproduction, including hormones (Robinson, 1987; Sullivan et al., 2000), yolk proteins (Amdam et al., 2004; Ihle et al.,

2010), and even the whole ovary (Wang et al., 2010; reviewed by Amdam and Page, 2010), affect behavioral transitions. Thus, worker behaviors are hypothesized to have evolved from reproductive traits exhibited by a solitary ancestor. The reproductive ground plan hypothesis (RGPH) (West-Eberhard, 1987, 1996; Amdam et al., 2004; Amdam and Page, 2010) argues that, instead of evolving a new regulatory infrastructure, regulators of development and behavior were co-opted from the solitary ancestral state, decoupled from reproduction, and utilized to control social behavior in functionally sterile individuals.

Some of the major components likely co-opted in this transition are the endocrine regulators of reproductive processes. Juvenile hormone (JH) and ecdysteroids are the primary hormonal drivers of reproduction in many insects (Lafont et al., 2005; Raikhel et al., 2005), but also play an important role in regulating social behaviors in functionally-sterile social insect workers (Hartfelder, 2000; Bloch et al., 2000; Brent and Vargo, 2003; Brent et al., 2006; Amdam and Page, 2010). In addition to these systemic hormones, the ovary itself can play a role as a regulator of insect behavior. In mosquitoes, ovarian factors help control host-seeking behaviors (Klowden, 1997), while in *Drosophila*, the ovaries modulate sensitivity to stimuli that influence behavior (Flatt et al., 2008). Similarly, in honey bees, worker ovarian development is robustly linked to age of foraging onset (Page and Fondrk, 1995; Amdam et al., 2006; Wang et al., 2009; Amdam and Page, 2010), and plays a causal role in behavioral development (Wang et al., 2010). The

* Corresponding author. Current address: Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA.

E-mail address: adolezal@iastate.edu (A.G. Dolezal).

ovaries may influence worker behavior through differential production of ecdysteroids (Amdam et al., 2010) and expression of genes coding for hormone receptors and sensory perception (Velarde et al., 2009; Wang et al., 2009, 2010, 2012).

In *Pogonomyrmex californicus* harvester ants, behavioral biases occur in both queens and workers. Individuals showing a bias towards foraging behavior exhibit elevated levels of JH relative to those that stay within the nest (Dolezal et al., 2009, 2012). Foraging-biased workers also have decreased levels of ecdysteroids (Dolezal et al., 2012). However, the hormonal association appears to be more correlative than directly causal, suggesting that other factors may be involved. While a direct link between behavior and ovarian activity has not been addressed in the ants, ovarian morphology differs drastically between workers in different task groups in many species. The ovaries of young, nurse workers are typically well-developed and active, while those of old foragers are heavily atrophied. However, it remains unclear whether these differences are due to the large age differences normally found between nurses and foragers, or if task performance *per se* drives these differences (Hölldobler and Wilson, 1990). Based on the putative relationship between reproductive physiology, reproductive hormones and behavioral preference in *P. californicus*, we hypothesize that ovarian activity is linked to non-reproductive behavioral preferences, independent of age. Since ovarian atrophy is associated with chronological age-based foraging activity in ants (Hölldobler and Wilson, 1990), and *P. californicus* workers show decreasing levels of the ovarian-produced ecdysteroids after foraging initiation, we predict that decreased ovarian activity is linked to a preference for foraging, even when foraging is precociously stimulated via colony manipulation.

2. Methods

2.1. *P. californicus* worker observation and collection

P. californicus single cohort colonies were formed and observed as described in Dolezal et al. (2012). *P. californicus* colonies were partially excavated from field sites in Maricopa County, Arizona in early November of 2010, and the light-colored callow workers were collected. Cuticle pigmentation was used as a marker for worker age (Wilson, 1976; Seid and Traniello, 2006), and only the lightest colored, and thus youngest, workers were collected. Workers were brought back to the laboratory, counted, and approximately 200 were added to each of four experimental colonies by pouring them from a plastic container; no signs of fighting were observed. Host colonies were 1.5–2.5 years of age, and had approximately 300 workers each. After 2 days, all of the original, dark-colored workers were removed, leaving only the queen, eggs, larvae, and approximately 200 young, same-aged workers. Colonies were then observed 3–7 days per week, 2–3 times per day, for 5–10 min per observation. Foraging individuals were determined to be workers observed interacting with food items (seeds or sugar water-filled test tubes) or water, and were then marked on the abdomen with small dots from a Sharpie® paint pen. Once a worker had been observed foraging on three occasions (and thus had three paint dots), she and an in-nest worker (which had never been observed foraging) from the same colony were collected together. These collections continued for the duration of the experiment, and all workers were collected between the ages of 23 and 33 days. The experiment was ended at this time, as the populations of all colonies had dropped below approximately 25 workers.

To verify that any patterns found in single cohort colonies reflected natural phenotypes, workers from unmanipulated field colonies were also collected. Age of these workers was unknown, as all unmanipulated workers possessed fully-darkened cuticles, but

P. californicus workers exhibit age polyethism and typically transition to foraging as they chronologically age (Dolezal et al., 2012). Therefore, foragers were presumably older than nest workers. Foragers were collected at bait traps near mature nests, and in-nest workers were collected from the brood chambers of four nearby colonies.

2.2. *P. californicus* queen collection and observation

P. californicus queens were collected immediately after mating flights in San Diego County, CA, USA in July 2008. Queens were collected from a population where queens naturally form multiqueen associations (Johnson, 2004). After collection, they were housed and observed as described in Dolezal et al. (2009). They were marked on the abdomen and thorax with one of two colors using a Sharpie® paint pen; one queen of each color was then introduced into a soil-filled nest jar. The soil was watered in small quantities when it became observably dry, and the colonies were fed a restricted quantity of Kentucky blue grass seeds. Associations were observed for 15 min intervals four times per day for 15 days to identify behavioral biases. Queens were recorded as foraging when observed outside of the nest entrance searching for or handling seeds. Associations were classified as having a division of labor if 10 or more foraging events were observed and more than 80% of those events were performed by one queen. The queen performing the majority of the foraging was categorized as foraging-biased, while the one performing the minority of foraging was categorized as nest-biased.

3. Ovarian dissection, fixation, and staining

A total of 36 *P. californicus* queens and 44 workers were collected after observations, kept on ice, and dissected in PTW (1× PBS; 0.05% Tween-20) using a dissection microscope. The entire ovary of each individual was fixed in a solution of dimethylsulfoxide (DMSO, 20 µl), 4% paraformaldehyde (200 µl) and heptane (600 µl) for 20 min. After fixation, ovaries were washed three times in PBT (1× PBS; 0.3% Triton). For long-term storage, ovaries were then washed in increasingly concentrated methanol:PTW solutions (30:70, 50:50, 70:30), culminating in pure methanol. After completing sample collection, ovaries were rehydrated with three washes of decreasingly concentrated methanol:PTW solutions (70:30, 50:50, 30:70) culminating in pure PTW (Khila and Abouheif, 2008). Samples were then incubated in 1:1000 dilution of the DNA stain DAPI (4',6-diamidino-2-phenylindole) (Invitrogen, Carlsbad, CA, USA) for 10 min, washed three times in PTW to remove excess stain, and mounted on microscope slides using Vectashield (Vector Labs, Burlingame, CA, USA). The stained and mounted samples were imaged using a Leica SP2 multiphoton scanning laser microscope (Leica Microsystems, Buffalo, IL, USA) and a Nikon Fluphot inverted fluorescent microscope (Nikon Instruments, Melville, NY, USA).

3.1. Ovarian activity scoring

The insect ovary is made up of multiple tube-like filaments, called ovarioles, where egg development occurs. As the eggs mature, they increase in size and move towards the anterior of the individual (the basal end of the ovariole). After the oocytes reach a certain size and maturity, the eggs uptake large quantities of yolk proteins and are termed vitellogenic (reviewed in Raikhel and Dhadialla, 1992). Therefore, we classified developing oocytes into one of several types (described below) based on their number, vitellogenic status, and location in each ovariole in queens and

Download English Version:

<https://daneshyari.com/en/article/5921920>

Download Persian Version:

<https://daneshyari.com/article/5921920>

[Daneshyari.com](https://daneshyari.com)