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Queen pheromones out of context: a comment on Holman

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Holman's (2018) meta-analysis demonstrates that there is ample experimental evidence of fertility-correlated cuticular hydrocarbons (CHCs) affecting worker fecundity across social insects. We agree with this finding; however, in framing and discussing his results, he fails to properly consider biologically relevant behavioral data. Another concern we have is the way he represented our review on the topic and our experimental studies.

Smith and Liebig (2017) developed a hypothesis for the evolution of CHCs as queen pheromones across insect societies that integrates the evolutionary transition from behavioral to purely pheromonal regulation of reproduction. We incorporated evidence for variation in how CHCs function in reproductive communication from cue-like signature mixtures, to fertility signals with context-learning, to innate queen pheromones. Holman positions his meta-analysis as a test of our work by giving the impression that our review exclusively favors "the theory that fertility-related CHCs only affect worker fecundity when presented in the correct context." This was only one of 3 ways which we theorized fertility-related CHC function.

Holman (2018) states that our review "used 'vote counting' i.e. tallying studies that support, or did not support, a hypothesis." However, we clearly stated that our review was not comprehensive and had a "narrow focus" on "an outstanding problem involving seemingly conflicting experimental studies." We explicitly did the opposite of "counting" or "tallying" by singling out studies that presented conflicting data and building an inclusive view of this field that considered how CHCs have different functions across species and social organizations.

After singling out one hypothesis from our review, Holman finds no evidence for it, reporting "the great majority of experiments that presented individual fertility-related CHCs recorded, strong, statistically significant effects on fecundity" and dismissing the "few published non-significant results" that did not show an inhibiting effect on ovarian development by isolated CHC compounds "as false negatives." Our review never advanced the idea that CHCs are interpreted as fertility signals in a singular way, testable by tallying a consensus result.

To our surprise, Holman (2018) repeatedly dismisses behavioral evidence for context-specific recognition of fertility signals. He not only contradicts his own definition of queen pheromones as signals that "have a multitude of effects, including…eliciting submissive…responses," but also ignores fundamental concepts in social insect behavior with respect to the social regulation of reproduction. Our behavioral experiments (Smith et al. 2015 contains 4 behavioral experiments, not 1 as Holman writes) demonstrate that submissive behaviors were only triggered by fertility-related CHCs in a relevant CHC context. Holman considers this as "no direct evidence for context-dependent responses" because "submissive behaviour might not translate into differences in fecundity." The concept that submission is a ritualized response to dominance and is generally associated with reduced fecundity in social insect hierarchies is well established (e.g. Bourke 1988; Drews 1993). As further justification of the dismissal of these experiments, Holman claims that "the experiment was not performed blind" even though the methods state that all behavioral data in Smith et al. (2015) were collected from blind analysis of video recordings.

Holman (2018) also focuses on the sample size (n = 13) of Smith et al.'s (2015) fifth experiment, a null result that an isolated fertility signaling compound has no primer-effect of inhibiting worker reproduction. This experiment compares group-level responses to controls and treatments. Unfortunately, Holman conflates group-level responses (presence of eggs in a colony) with individual-level responses (ovarian status) within groups by treating sample sizes as equivocal. He concludes that a suitable sample size for this experiment would have been about 130 for each treatment group. However, group-level responses are often an average of individual responses, especially for an egg-laying. For example, Smith et al. (2015) measured 13 paired groups which consisted, on average, of 21 workers. Thus, this experiment tested an average of 273 workers per treatment. Furthermore, individual-level measures assume that a significant proportion of workers will develop their ovaries in response to the absence of a queen. This is not true for species in which egg-laying is taken over by one or a few individuals.

Finally, we agree with Holman (2018) that more work is needed on this topic. We are happy to see additional behavioral data emerging, such as a recent study in termites supporting the idea that perception of fertility signaling CHCs can be dependent on chemical context (Funaro et al. 2018).

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Received 5 April 2018; editorial decision 10 April 2018; accepted 14 April 2018; Advance Access publication 9 May 2018

doi:10.1093/beheco/ary065

Editor-in-Chief: Leigh Simmons

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