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研究課題名（和文）RNA matters: the role of transmissible RNAs for social and individual immunity

研究課題名（英文）RNA matters: the role of transmissible RNAs for social and individual immunity

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研究成果の概要（和文）：社会性動物では、フェロモンは生殖とタスクの分割の両方を制御します。 現在の理論は、社会性昆虫が昆虫の経路の共同選択から複数回進化したことを示しています。 孤独な昆虫モデルを使用して、私はフェロモンがコオプテーション仮説を裏付ける同様のトランスクリプトミクス信号を作成するかどうかを調べました。 キイロショウジョウバエのトランスクリプトミクスと生理学を他の種のフェロモンに測定しました。 いずれの場合も、私は強い生理学的反応、つまり卵巣のサイズの縮小を観察しました。 しかし、保存された経路や遺伝子は検出できませんでした。 これは、フェロモンを介した社会性が収斂進化の結果であることを強く示唆しています

研究成果の学術的意義や社会的意義
The study of mechanisms and pathways enable the evolution of social living is important step for a broader understanding of evolution of life.

研究成果の概要（英文）：In eusocial animals, pheromones control two distinct aspects of the division of labour: reproduction and division of tasks amongst workers. It has been suggested that eusociality arose on multiple events from pheromone-mediated co-option of pathways present in solitary insects. Using a solitary insect model, I sought to determine if these similar transcriptomic signals resulted from the co-option of similar pathways or varying pathways subject to convergent evolution. I measured the transcriptomic and physiological response of Drosophila melanogaster to pheromones from bumblebees, honey bees, and termites. In each case I observed a strong physiological response - the reduction of ovary size. However, I could not detect any conserved pathway or genes acting as a primer for eusociality. This strongly suggests that pheromone-mediated eusociality is the result of convergent evolution.

研究分野：Evolution

キーワード：Eusociality　Pheromone　Social evolution　Honey bees

１．研究開始当初の背景

When studying the evolution of life on earth, it is possible to observe the presence of a few drastic changes in the complexity on which organisms are organised, termed "Major Evolutionary Transitions". Each evolutionary transition transformed the evolutionary process as evolutive pressure was vastly different between ancestral and derived states. They also required considerable innovation and changes in physiology, gene regulation and activation. Such as the transition from Prokaryotic cells to eukaryotic cells, unicellular to multicellular organisms and from solitary to group living. As such, the investigation of the mechanisms that enable these transitions (i.e. physiological, genetic, ecological, transcriptional) has held a prominent status amongst scientists.

The evolution of social living, sociality, is perhaps one of the most studied transitions, especially in cases where animals display extreme forms of altruistic behaviour, such as forfeiting personal reproduction, as observed in eusocial animals. Eusociality if considered the highest form of organisation of sociality and is defined by cooperative brood care, overlapping generations within a colony, and a division of labour into reproductive and non-reproductive groups. Division of labour is one the most studied traits in eusocial insects, as it is highly dependent on reproductive altruism when most of the group members trade their reproduction to increase (i.e. workers) the reproductive success of another individual (i.e. queens). The control of such reproductive state varies, determined by nutrition or chemical control. Higher eusocial insects, such as wasps, ants, honeybees and termites, evolved independently of the type of chemical control, the use of pheromones, that can control behaviour (e.g. cooperative brood care) and physiology (e.g. ovary development) of workers.

Complex traits, rarely (if ever) appear from nothing. They evolve from the co-option of preexisting genes and gene networks. As a result, if a complex trait evolves from the co-option of existing genes, the modification of upstream factors within the network in an ancestral organism could generate modifications comparable to the derived structure. This seems to be the case of eusocial animals. Comparative analyses of gene expression between different eusocial species indicate that eusocial traits evolved from the co-option of pathways present in solitary ancestors. However, cross-species transcriptomic comparisons are complicated due to the evolutionary distances between eusocial insect species as inter-species gene orthologs may have vastly different functions. Therefore, differences or similarities in expression patterns between two highly divergent species can be highly misleading. Thus, pheromone-induced transcriptomic changes reflect extensive evolutionary optimisation of affected networks. One approach to overcome this problem is to investigate transcriptomic changes induced by varying pheromones in a single species. This permits the study of conserved and co-opted transcriptomic changes and identifies molecular signatures of these rare evolutionary events.

２．研究の目的

In this research, I investigated how pheromones alter physiology and gene expression in solitary individuals. Using Drosophila melanogaster, I examined if pheromones regulating eusociality evolved from the co-option of similar pathways present in solitary ancestors. Specifically, I investigated whether distinct pheromones have a similar profile, genetic and physiological, across multiple instances in which it evolved, as previously suggested. I present a comprehensive transcriptome dataset set investigating gene expression associated with the reproductive division of labour. Specifically, I focus on expression changes induced by queen pheromone produced by termites (*Reticulitermes speratus*), bumblebees (*Bombus terrestris*) and honey bees (*Apis mellifera*) and brood pheromone produced by honey bee larvae. I leveraged this data set to perform, to my knowledge, the most up to date comparison of differentially vs distinct (i.e. non-homologous) expressed genes associated with the maintenance of eusociality. These results were further developed into an online application that aims to facilitate further exploration of results and can be readly accessible - https://bxuom8-mariana-velasque.shinyapps.io/Eusocial_pheromone/.

３．研究の方法

I used the fruit fly, *D. melanogaster*, to investigate the hypothesis that eusociality evolved multiple times by co-opting existing regulatory pathways present in a solitary ancestor. I achieved this by studying the pheromone genetic signature of fruit fly exposed to pheromones from three different eusocial species: honey bee queen mandibular pheromone (QMP), bumblebee queen pheromone (Bu), termite queen pheromone (T) and a pheromone produced by honey bee larvae, the brood pheromone (Br). To simulate conditions present in eusocial colonies, when they are exposed to both brood and queen pheromone, I also added a group with a mix of queen and brood pheromone (QMP + Br).

Flies were raised on standard Bloomington media at 24 °C under a 16h light / 8h dark cycle in 50 mL vials. The D. melanogaster strain Canton-S, a generous gift from the Van Vactor Unit, was used in all experiments. Each experimental vial contained 10 each of male and female approximately 5 day old flies and each treatment consisted of 10 vial replicates. Bloomington fly food was made and allowed to cool to >60°C before mixing in each of the phenomenon treatments at a 1 in 150 ml concentration. Ten millilitres of supplemented food was added to each vial and left to dry for 24 hours. Vials were kept at 25°C and under a powerful extractor to prevent cross-contamination due to pheromone volatility. Adult flies were left on the vial for four days to lay eggs and then removed and discarded. Virgin females were collected and allowed to mature for 5 days in vials containing the aforementioned food with the corresponding treatment pheromone.

Ovary collection for measurement was performed in triplicate and on the same day to prevent temporal changes in physiological measurements of ovaries. Ovary

image identities were encoded such that they could be measured blind to reduce bias. Measurements were performed using ImageJ2.

For RNA sequencing, I randomly selected 4 vials and pulled 5 ovaries per vial. RNA extraction was performed with an in-house modified protocol. Libraries were prepared using NEBNext® Ultra™ II Directional RNA Library Prep Kit (E7760L) for Illumina as instructed by the manufacturer.

The complete analysis pipeline and associated results are publicly available at https://github.com/marivelasque/eusociality_evolution. This also includes code for the Shiny application that can be assessed at https://bxuom8-mariana-velasque.shinyapps.io/Eusocial_pheromone/. RNA data as assessed using FASTQC and trimmed with Trimmomatic 0.38. Transcript abundance was calculated with RSEM/bowtie2. Mapping and abundance calculations were performed against the D. melanogaster genome assembly BDGP6 (release 89). Differential expression analysis was performed using edgeR, limma-voom and DEseq2. All p-values were adjusted to control the false discovery rate using the Benjamini-Hochberg method. Threshold of significance was defined by $P<0.05$. In addition to the differential gene expression analysis, I also used two complementary approaches to investigate changes in gene network mediated by eusocial pheromones, eighted Gene Co-Expression Network Analysis WGCNA and dcanr. Threshold of significance was defined by $P<0.05$. Gene Ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) were estimated using topGO and clusterProfiler respectively to identify enriched terms.

To ensure pheromonal induced changes in the fruit fly transcriptome is comparable to eusocial insects, I also compared the network conservation of eusocial insects, *Apis mellifera* and *Bombus terrestris*, and fruit flies exposed to pheromone. This comparison was made using preservation statistics in a WGCNA integrated function (modulePreservation). Threshold of significance was defined by $P<0.05$. No publically available pheromone termite treatment data were identified.

4．研究成果

I identified a significant change in ovary size across all pheromone treated D. melanogaster relative to solvent only controls (Repeated measures ANOVA $F_{6,180}$, $p < 0.0001$; Figure 1; Tables S1 – S3). I did not find significant differences in ovary size between the two controls ($p = 1.0$; Figure 1). Although pheromone treatment strongly inhibited ovary size, their effects varied in strength. Bumblebee and Termite pheromone had similar ($p = 1$) severe pheromone-mediated ovary reductions of 0.32 mm2 and 0.35 mm2 (mean; n=10) respectively. Pheromone treatments from A. mellifera queen mandibular pheromone (QMP), Brood and the QMP and Brood mix pheromone induced similar reductions on ovary size (QMP and Brood vs QMP $p =1$; QMP and Brood vs Brood $p =1$; QMP vs Brood $p = 0.064$) of 0.51 mm2, 0.65 mm2 and 0.59 mm2 (mean; n=10) respectively.

Using conventional differential gene expression analysis, edgeR, I identified no overlap between pheromone treatment groups. Within each treatment, I identified similar numbers of differentially expressed genes with brood pheromone treatment being elevated compared to other treatments. There was no shared core of pheromone response genes and negligible inter-treatment overlap (Figure 2B). As I was unable to identify a core of differentially expressed genes, I investigated if pheromone-mediated changes in gene expression affected reproductive physiology through similar cellular processes. I compared gene enrichment of pheromone induced changes in the transcriptome using GO (gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) annotations. Although I identified some inter-treatment pathway enrichment, again I failed to identify a core pathway/pathways.

Eusocial pheromones originated at least 100 Mya and evolved independently in multiple species. Their evolution and signal can be well traced across three groups: Blatoidea, Hymenoptera and Coleoptera, causing virtually a similar phenotype in all groups. The independent evolution of pheromones, the high conservation with their eusocial counterpart, combined with these results (i.e. pheromone treatment causing the similar ovary reaction in flies with no overlapping gene network and pathways) suggests pheromones are not a product of gene co-option, but the result of convergent phenotypic evolution. Producing a similar phenotype (inhibition of ovary development) through a different pathway in the species on which it evolved.

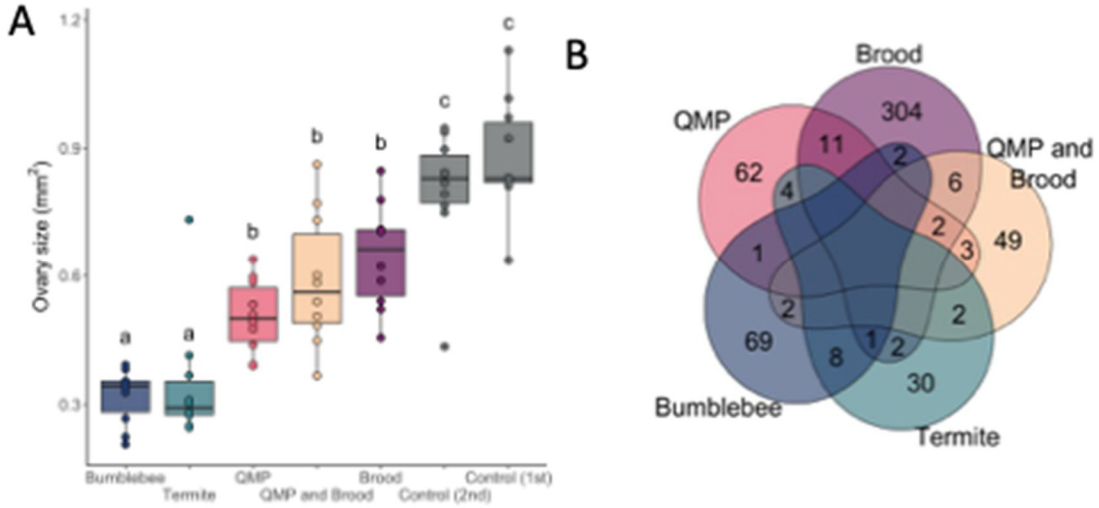

Figure 1 – (A) Pheromone treatment resulted in reduced Drosophila melanogaster ovary size and (B) D. melanogaster gene expression.

5．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

1．著者名 Denton Jai A.、Koludarov Ivan、Thompson Michele、Bryk Jaros?aw、Velasque Mariana	4．巻 12
2．論文標題 Honeybee Cognition as a Tool for Scientific Engagement	5．発行年 2021年
3．雑誌名 Insects	6．最初と最後の頁 842～842
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/insects12090842	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Velasque M.、Tan Y.、Liu A.W.、Luscombe N.M.、Denton J.A.	4．巻 2022
2．論文標題 Suppressed eusocial reproduction supports evolutionary convergence over co-option	5．発行年 2021年
3．雑誌名 bioRxiv	6．最初と最後の頁 1-1
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2021.07.11.451940	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関