Report

Cell Systems

Self-organization of plasticity and specialization in a primitively social insect

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[2,13,15,18,*]

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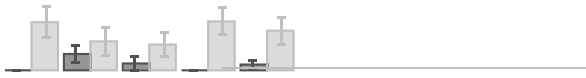

(Figure 1E). This observation is further supported by an increase in the correlation of queen gene expression profiles of workers in the D4 phase with queens in control nests (Figure 1F), consistent with the coordinated change of queen gene expression observed after queen removal in another species (Taylor et al., 2021). Taken together, these observations show that at the transcriptome level, queen removal rapidly causes a phenotypic switch to queens in all workers. However, this loss of worker phenotypes after queen removal is transient as individuals collected from 3 nests having already recovered their steady state (34 individuals, D14 phase) show gene expression profiles similar to unmanipulated workers (Figure 1F).

Understanding how the interplay between different scales of biological organization can give rise to specialization and plasticity requires understanding how perturbations on all scales are propagated across the different layers of biological organization. As this is experimentally unfeasible, our strategy is to derive a theory that predicts the multi-scale response of the society to perturbations. Such a theory would constitute a complete biophysical understanding of the multi-scale nest dynamics. As a first step, following the approach of statistical physics, we sought to define the simplest model compatible with the experimental data. In particular, in order for this model to be unsensitive to the partially unknown details of the complex processes underlying the regulation of the society, we did not assume any non-linear terms unless motivated by experimental observations. To begin deriving a biophysical description of the nest dynamics, we describe the molecular state of insect by a single degree of freedom, . This is supported by the observation that different kinds of molecular regulation respond to queen removal on similar time scales (hours), by our observation that the expression levels of different queen genes are correlated and by earlier observations in which and were upregulated within a few hours under hormonal influences resulting from the queen's absence or through induction (Edinger et al., 1997; Hamilton et al., 2016; Röseler, 1977; Röseler and Röseler, 1978). Importantly, our results do not depend on specific assumptions on the dynamics of particular molecular processes. Although therefore represents a coarse-grained description of complex molecular states, we will, for specificity, henceforth refer to as the concentration of queen gene products as an important instance of such states. The time evolution of the probability that a randomly chosen insect has a concentration of queen gene products , (,), is given by changes (so called probability fluxes) due to molecular processes, , and feedback by the colony level, , v (,) = + . Our RNA-seq experiment shows that in the absence of the queen, individuals constitutively express

8221TD.9(products);-342(as)-344.8(an)-3300.o273cf.2.25610TD[(Eding335195

with control nests (Figure 4G). To verify that this trend of demethylation was not linked to genetic differences between nests, we validated this result by mass spectrometry by analyzing the global level of DNA methylation of 5 nests from which a subset of individuals was taken before and after queen removal. In each case, we observed a consistently lower rate of DNA methylation in individuals collected during the eggless phase, in which aggressive behaviors are highest (Figure 2B and Figure S5A), confirming the association of DNA methylation levels with the stability of the nest.

Although these observations will require further investigation, in particular for the identification of the mechanisms leading to the depletion of DNA methylation (Figures S5B and S5C) and to establish the reversibility of DNA methylation erasure in -, our observations support a role of DNA methylation in stabilizing the society against strong fluctuations.

DISCUSSION

A combination of experimental and theoretical approaches shows that uses antagonistic dynamics on different spatial scales to distinguish between molecular- and colony-level perturbations, thereby achieving robustness to the former and plasticity to the latter. In our approach, we combined molecular profiling using multi-omics with colony-level video recordings in a way that correlates observations on the molecular, individual, and nest scales to inform a biophysical model and a theory that predicts the propagation of perturbations through multiple scales of organization in societies. In our experiments, we studied colonies of in their natural habitat during field work expeditions in Panama. Although studying a social insect in its natural environment limits the use of molecular perturbation techniques available in laboratories, it allowed us to perform behavioral perturbations under natural environmental fluctuations of nests.

Because of its complexity, a model comprising an accurate description of the biological complexity of the processes regulating the insect society would not allow gaining analytical insight into the response of the society to perturbations. We therefore incorporated the minimal set of assumptions that followed directly from our experimental observations. As the behavior of dynamical systems is governed by the stability of their attractors, our main conclusions in Figure 3 are robust with respect to the addition of biological complexities going beyond these assumptions. Importantly, in the spirit of statistical physics, variables constituting the model are therefore to be interpreted as coarse-grained descriptions of the complex processes regulating the society. Our theoretical work then allowed us to fully understand the response of the society on all scales to perturbations on the molecular and societal scale and, consequently, the self-organization processes underlying the simultaneous regulation of specialization and plasticity. These results show how societies of primitively social insects can control how fluctuations propagate across scales of biological organization to perform specific functions. Our results also suggest that DNA methylation seems to play an unanticipated role in regulating the stability of the society at the colony level. Our work demonstrates that correlated measurements across scales can give qualitatively new insights into the mechanisms underlying self-organization of biological systems (Figure 4H). Our approach may be more widely applicable to other biological systems of interest and expanded to more complex societal structures (Sasaki et al., 2016). Our work might also help to understand evolutionary processes on much longer time scales (Menzel and Feldmeyer, 2021), as it provides a unified framework for studying the transition from solitary insects to insect societies.

STAR + METHODS

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STAR + METHODS

KEY RESOURCES TABLE

RESOURCE AVAILABILITY

Lead contact

experiment and none of them had developed mature ovaries during the experiment. All nests were monitored daily in order to mark every new emergent worker and censused every other evening to know the entire nest population. All emergent workers of the - species are mated in early life and then have the potential to become a queen (Turillazzi and West-Eberhard, 1996). The queen was identified by removing an egg and observing who replaced it, as well as from behavioural observations and census data. 26 nests were monitored for three to nine weeks and female wasps representing the different stages of the queen succession were collected. 8 nests were collected unmanipulated (Control phase). Phenotypic reprogramming was induced by removing the queen and an egg in 18 nests and monitored every day to detect any new egg layers. 7 nests were collected before an egglayer appeared (Eggless phase). As soon as a new egg appeared the eggless phase was considered to be over. The identity of this new egg layer was determined over the following day by behavioural observations and then 5 nests were collected (D1 phase). A further 3 nests were monitored and collected 4 to 5 days after the first egg laying occurred (D4 phase). Finally, 3 nests were monitored until 2 weeks after egg laying (D14 phase). All collected nests had similar stage of development (35.6 ±14 days post-emergence) and comparable size (15.6 ± 7.2 individuals) and no sign of parasites or disease. Wasps were collected directly off their nests individually with forceps during the active hours of the day. Their heads were cut off and immediately placed in RNA later solution (Ambion) incubated at 4C overnight to ensure that solution penetrates the brain and kept at -20°C until the dissection of brains. Their bodies were stored in 80% ethanol and kept at -20°C until dissection of ovaries.

Belonogaster juncea

Three nests of and their 11 individuals were collected in March 2013 in Ebolowa, Cameroon (2°55′N 11°9′E). Collection, storage and reproductive state assessment of all individuals were done similarly than for .

Ovaries dissections

Reproductive state was assessed for 385 wasps by measuring the first and biggest egg at the entrance of the oviduct. A mature egg has a size of 1.5 mm, which corresponds to the smallest egg size observed and associated with egg-laying. Ovary size was used a proxy for queen gene expression (and -3) (Röseler et al., 1983; Giray et al., 2005; Röseler, 1977).

Video analysis

cleaning between steps were performed with the AMPure XP system (Agencourt) to select DNA fragments between 250 bp and 500 bp. Paired-end libraries were sequenced on HiSeq 2500 Illumina platform.

RNA-seq libraries were sequenced on the Illumina HiSeq platform using the default RTA analysis software. RNA-Seq data were trimmed with Trim Galore (v0.4.1, default parameters) and mapped to the genome assembly GCF_001313835.1 using TopHat v2.0.12 as previously described in (Patalano et al., 2015). Strand specific quantification was performed using RNA-seq pipeline in Seqmonk software Version 1.39.0 (www.bioinformatics.babraham.ac.uk/projects/seqmonk/). To normalize across nests size factors were calculated using the DEseq2 package in R and log transformed with only orientated sam-

Biophysical modelling and theory

A detailed and rigorous derivation of the theoretical approach is given in the supplemental theory. Briefly, we defined the minimal stochastic model capable of describing the experimental phenomenology. The evolution of the distribution $(\{ , \},)$ of the number of queen gene products, , and the state of queen gene repression, , in each individual at a given time , is given by

$$\frac{}{} (\{ , \},) = \sum_{=1}^{+1} \Big\{ \mu(1 -)[(\{ - 1, \},) - (\{ , \},)] + \delta[(+ 1) (\{ , \},) - (\{ , \},)]$$
$$+ G( )(1 - 2) (\{ , 1\},) + \omega(2 - 1) \sum_{\neq} (\{ , 0\},) \Big\},$$

where the first two lines account for the production and degradation of queen gene products and the last line contains the terms