



## **Comparative transcriptomics reveals the ancestral brain gene-network regulating caste differentiation in ants**

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A superorganismal caste system with physically differentiated queens (inseminated) and workers (life-time-unmated) originated only once in the common ancestor of all ants. This implies that caste-specific gene-expression should have retained homologous ancestral characteristics, but the identity of the genetic regulatory network (GRN) reflecting the origin of superorganismality in ants has remained obscure. We analysed the caste-specific brain transcriptomes of five ant species from three subfamilies and show that the ancestral network for caste-specific gene regulation has indeed been maintained, but that signatures of common ancestry are obscured by later lineage-specific modifications. Adjusting for such differences, we identified a core set of genes that consistently display similar directions and degrees of caste-differentiated expression across ant species. Most of these genes have not been reported before as being involved in caste differentiation. We also show that these core caste-regulatory genes continue to exist in the genomes of two ant species that secondarily lost the queen caste, but that their expression differences in reproductive and sterile phenotypes are minor and similar to expression differences between egg-laying and sterile females of paper wasps where differentiated castes never evolved. Many of the core caste-regulating genes of ants also have caste-differentiated expression in the honeybee, but the directions of caste-expression-bias of these genes between ants and honeybees are uncorrelated. This is consistent with both lineages having used overlapping sets of toolkit genes to independently evolve irreversible transitions to superorganismality with a specialized fertile queen caste and a life-time unmated worker caste.