

The making of a social insect – the regulatory architectures of social design

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The origins of social behavior in insects are sought by mechanistic and evolutionary approaches with the common goal of understanding the foundations of social life. The transitions from solitary to social living are studied at multiple levels of analysis ranging from molecular genetics and genomics (e.g., Robinson et al. 2005; Page et al. 2006) to evolutionary theories of inclusive fitness and altruism (e.g., Wilson and Hölldobler 2005; Foster et al. 2006). A fundamental focus of this research is to explain how complex social phenotypes can evolve from ancestral solitary forms.

A theoretical framework addressing this question was developed by West-Eberhard (the ovarian ground plan hypothesis: 1987; 1996). It proposed that the ovarian cycle, as a sequence of co-regulated physiological and behavioral events, provided a mechanistic basis for insect social evolution. The idea was consistent with correlations between ovarian physiology (developed vs. undeveloped ovary) and behavioral phenotype (queen or non-worker behavior vs. worker behavior, respectively) in primitively social wasps. Pleiotropic trait-associations within the worker castes of a wide variety of eusocial wasps, ants and bees further supported the idea: slight ovarian development was generally interlinked with seemingly derived reproductive activities such as cell initiation, brood-rearing and production of trophic eggs, whereas individuals with undeveloped ovaries performed tasks outside the nest such as foraging. West-Eberhard's hypothesis implied that these trait-correlations were signatures of a fundamental principle in social evolution: the segregation of queen and worker forms and subsequent division of labor between workers relied on evolutionary co-option of phases from the ancestral ovarian cycle.

The ovarian ground plan hypothesis did not address how social behavior was regulated at the molecular level, and it was not directly tested. The systemic hormone juvenile hormone (JH), however, was suggested as a likely coordinator of the pleiotropic trait-associations between ovarian physiology and behavior (West-Eberhard 1996). JH affects reproductive maturation including behavior in many solitary insects (reviewed Flatt et al. 2005). In the honey bee (*Apis mellifera*), the best-studied eusocial insect in terms of hormonal regulation, JH is part of a feeding-sensitive endocrine switch that regulates the queen-worker differentiation. Also, JH has pleiotropic effects on honey bee worker physiology (e.g., Rutz et al. 1974) and behavioral ontogeny (e.g., Jaycox et al. 1974; Sullivan et al. 2003). Yet, the honey bee did not readily exemplify the proposed link between reproductive physiology and complex social behavior. Worker bees do not show ovarian activation concomitant with brood-rearing, and JH appears to have lost gonotropic functions in the adult stage (reviewed by Hartfelder 2000). This loss of function was further suggested to have facilitated the evolution of a novel role of JH in the control of age-polyethism in workers bees (Robinson et al. 1992; Robinson and Vargo 1997). Thus, one might conclude that complex social behavior in honey bees is a derived trait associated with the origins of advanced eusociality in the lineage, and not with the origins of sociality per se (Michener 1974).

Molecular analysis of the division of labor in honey bees, conversely, supported the general assumption that complex social behavior is influenced by pathways inherent from solitary life. Results from research groups led by Robinson and Sokolowski showed that division of labor between nurse bees engaging in brood rearing and foragers collecting pollen and nectar in the field are affected by the genes *Amfor* and *AmMvl* that in *Drosophila melanogaster* regulate feeding-related behavior (Ben-Shahar et al. 2002; Ben-Shahar et al. 2004). These findings suggested that genes involved in solitary behavior are used for social behavior, and that molecular

insights from solitary behavior can be used to search for candidate genes for more highly derived patterns of social behavior (Robinson et al. 2005).

Resolving evidence in this debate emerged from studies of the gene *vitellogenin*. The vitellogenin protein is a conserved egg-yolk precursor with dynamic expression levels that diverge between the temporal worker castes of honey bees (Engels and Fahrenhorst 1974). In most solitary insect females, vitellogenin is synthesized exclusively during the active reproductive phase. Therefore, the presence of high levels of vitellogenin in functionally sterile nurse bees and low levels in foragers were not immediately understood (Rutz and Lüscher 1974). Amdam et al. (2003a), however, showed that nursing worker honey bees utilize vitellogenin as a source of the proteinaceous jelly they feed to larvae. This finding implied not only that worker bees show task-associated expression of a reproductive protein, but also that vitellogenin is utilized in the honey bee worker caste as it is in hymenopteran species that rely on trophic eggs for larval nourishment (Amdam et al. 2003a). Vitellogenin, therefore, could provide an explanatory bridge between the social behavior of worker bees and the ovarian cycle of an ancestral solitary form (Amdam et al. 2004).

This proposition was tested in the selected pollen-hoarding strains of Page and Fondrk (1995). Amdam et al. (2004) found that the high pollen-hoarding strain, which preferentially collects and stores pollen, is characterized by elevated vitellogenin levels; and the low pollen-hoarding strain, which is biased toward collecting and storing nectar, is characterized by low vitellogenin levels. Amdam and Page hypothesized that elevated vitellogenin levels correlated with pollen hoarding because collection of pollen for storage in the nest was an ancestral reproductive behavior. They referred to the underlying mechanistic architecture as a reproductive ground plan: a pleiotropic regulatory gene-network that coordinated the physiological, behavioral and sensory components of an ancestral reproductive cycle. This regulatory architecture had not been disassembled during social evolution. Rather, it had been exploited to produce a division of labor between workers: pollen foragers displayed the ancestral maternal character state of solitary species, whereas nectar-foraging bees displayed a non-reproductive phenotype tuned for self-maintenance and nectar feeding. This hypothesis was later supported by the finding that high pollen-hoarding strain bees have larger ovaries (a higher number of ovariole filaments) and a stronger propensity for ovarian activation and egg-laying than low pollen-hoarding strain bees (Amdam et al. 2006a). The association between ovariole number and foraging preference for pollen or nectar was also confirmed in unselected (or wild type) worker honey bees.

Yet, even with this first experimental demonstration of a role of ancestral reproductive networks in regulation of complex social behavior, the molecular anatomy of the underlying architecture, or ground plan, was not explained. The development of an RNA interference (RNAi) protocol for the *vitellogenin* gene (Amdam et al. 2003b), however, allowed for functional studies of the causal relationships between this reproductive element and honey bee social behavior. These experiments showed that *vitellogenin* gene activity pleiotropically affects sensory responsiveness (Amdam et al. 2006b), onset of foraging behavior, and foraging preference for pollen or nectar in worker bees (Nelson et al. 2007). Also, RNAi revealed that *vitellogenin* interacts with JH through a positive feedback mechanism that enables the two components to mutually suppress each other (Guidugli et al. 2005). This regulatory architecture, which appears to build on reproductive control-mechanisms of solitary insects, shows divergence between high and low pollen-hoarding strain bees – possibly explaining developmental, physiological and behavioral differences between them (Amdam et al. 2007).

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