Molecular evolution of juvenile hormone esterase-like proteins in a socially exchanged fluid

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Socially exchanged fluids, like seminal fluid and milk, present a direct and effective means through which an organism can influence conspecifics. Given the high stakes of behaviors wherein fluids are exchanged, the contents of these fluids can be subject to powerful selection pressures that can lead to novel functions. Our previous research indicated that when Carpenter ant workers (*Camponotus floridanus*) provide nutritive trophallactic fluid to developing offspring, they also transfer a key developmental regulator, juvenile hormone.
To better understand the prevalence and origin of this exchange, we have explored the molecular evolution of a developmental regulatory enzyme family (juvenile hormone esterase, JHE) whose member-proteins constitute more than 18% of the total endogenous protein in this species' trophallactic fluid. We paired proteomic, behavioral, and small molecule measurements in a selection of species with phylogenetic and positive selection analyses of 30 species. Relative to other formicine ants (*Formica, Lasius, Cataglyphis*), *Camponotus* esterases have undergone positive selection, sustained multiple duplications, and have changed localization. To determine whether these esterases might function in vivo, we exposed the trophallactic fluid to a JHE-specific inhibitor by adding it to workers' food. The inhibitor altered the likelihood of pupation in the larvae reared by these workers in a manner similar to administering juvenile hormone. Together, these findings suggest JHEs may have undergone neofunctionalization in this genus, expanding from an intra- to inter-individual developmental regulator involved in the social control of larval development.