THE PROTEIN PHEROMONE FAMILY OF E. PETZI, A PSYCHROPHILIC AND EARLY BRANCHING EUPLOTES SPECIES

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Euplotes species are valuable for the study of the structural and functional biology of water-borne protein pheromones that cells constitutively synthesize and use in intra-specific chemical communication. We have recently devoted particular attention to the pheromone family of the "cold-loving" (psychrophilic) species *E. petzi* which dwells in the freezing Antarctic and Arctic coastal sea waters, and forms, together with *E. sinicus,* the earliest branch of the *Euplotes* phylogenetic tree. From cultures of genetically distinct strains, we have isolated and sequenced four *E. petzi* pheromones. With respect to the known pheromones from *E. raikovi, E. octocarinatus, E. nobilii* and *E. crassus,* the *E. petzi* pheromones are smaller (32 amino acids) and richer in Cys residues (eight) located in strictly conserved positions. These residues are predicted to form four intra-chain disulfide bridges, which suggests a compact globular fold of the molecules. However, the NMR solution structure determined for one of the *E. petzi* pheromones challenges this hypothesis. The structure consists of one more extended eight-residue alpha-helix and one smaller four-residue helix, and shows large polypeptide segments devoid of regular secondary structures. Pheromones from other *Euplotes* species which live in temperate waters and branch later than *E. petzi* in the *Euplotes* phylogenetic tree are known to be characterized by a three-helix fold and unstructured regions of comparatively limited dimensions. In the light of this knowledge, we can thus draw two distinct conclusions from our findings. The first, of phylogenetic nature, is that the structural evolution of the *Euplotes* pheromones involves an increase in size and complexity. This is in line with the smaller and simpler organization that also the macronuclear *E. petzi* pheromone genes show with respect to their homologues in other Euplotes species. The second conclusion is that the extended unstructured regions of *E. petzi* pheromones are likely correlated with an increased flexibility of the molecular backbone and, hence, reflect a common feature of protein cold-adaptation. In this regard, further insights will be obtained by ongoing experiments which aim to assess the unfolding and refolding properties of *E. petzi* pheromones when exposed to increased temperatures and variations of other environmental parameters.