Isaksson C. 2007. Ecology and physiology of carotenoid coloration in great tits, *Parus major*

Abstract- The springboard to this thesis was our discovery that urban great tits, Parus major, in the city of Göteborg (southwest Sweden) had a paler yellow carotenoid-based ventral plumage and increased levels of oxidative stress compared to great tits living on Onsala (rural environment) (I). One possible explanation for these results is an allocation conflict between the dual functions of carotenoids, as plumage pigments and as physiologically important antioxidants, suggesting that plumage coloration may serve as a non-invasive biomarker for oxidative stress. Antioxidants protect the body from Reactive Oxygen or Nitrogen Species (ROS or RNS) and both internal and external factors can increase these levels (e.g., through metabolism, infection, and inhalation of urban anthropogenic pollution). However, the antioxidant efficiency of carotenoids in vivo is poorly understood and the often presumed link between carotenoid pigmentation and health has recently been suggested to be a "red herring", i.e., correlated with other health improving mechanisms. In this thesis, I explore ecological and physiological factors affecting plumage coloration in great tits, primarily nestling carotenoid coloration. Factors explored include seasonal and environmental variation in external and internal carotenoid availability, and the role of carotenoids as free radical scavengers during oxidative stress and its effects on plumage pigmentation.

In a cross-fostering experiment, we showed that much of the variation in nestling plumage pigmentation derives from variation in post-hatching parental effects, in particular paternal effects with a relatively minor contribution from maternal carotenoid allocation to the egg yolk (II). However, neither carotenoid content of egg yolk, nor the carotenoid intake per offspring via caterpillars (*Lepidoptera*) differed between urban and rural environments (II, IV). The similarities in post-hatching carotenoid availability resulted from a higher mass and abundance, but lower carotenoid concentration, of caterpillars in the urban habitat (IV). However, a more qualitative (compositional) difference was found, with rural yolks and caterpillars containing relatively more zeaxanthin than lutein (III, IV).

Internal carotenoid availability for adults showed seasonal, environmental and sexual differences (V). If some of these differences depend on the use of carotenoids as antioxidants, a positive relationship between Total non-enzymatic Antioxidant Activity (TAA) and plasma carotenoids should exist. However, TAA was not related to carotenoid pigmentation (VI). Furthermore, experimental exposure to an oxidative stressor suggested that carotenoids are inefficient antioxidants in vivo (VII). Oxidative stress is therefore unlikely to provide a proximate link between individual health and pigmentation (VII). The final paper provides a detailed explanation of the plumage reflectance measurements used throughout the thesis. We also show that there is no sexual dimorphism in carotenoid incorporation into feathers, which suggests that the yellow breast of great tits is not a sexually selected character (VIII).

The main conclusions of this thesis are; (1) rearing environment and pre-hatching maternal effects influence nestling coloration, rearing environment being most important; (2) urban environment has a negative impact on pigmentation, condition and stress level; (3) but there is no direct link between oxidative stress and pigmentation. This is explained by a minor role of carotenoids as antioxidants in vivo. Instead, the results in this thesis suggest that the paler urban plumage reflects a limited external access to zeaxanthin (which causes a more yellow plumage than lutein) in the urban environment. Further studies are needed to evaluate this hypothesis and to test whether carotenoid pigmentation could act as a biomarker of environmental stress.