Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

Cuticle formation and pigmentation in beetles

Mi Young Noh[1], Subbaratnam Muthukrishnan[2], Karl J Kramer[2] and Yasuyuki Arakane[1]

Adult beetles (Coleoptera) are covered primarily by a hard exoskeleton or cuticle. For example, the beetle elytron is a cuticle-rich highly modified forewing structure that shields the underlying hindwing and dorsal body surface from a variety of harmful environmental factors by acting as an armor plate. The elytron comes in a variety of colors and shapes depending on the coleopteran species. As in many other insect species, the cuticular tanning pathway begins with tyrosine and is responsible for production of a variety of melanin-like and other types of pigments. Tanning metabolism involves quinones and quinone methides, which also act as protein cross-linking agents for cuticle sclerotization. Electron microscopic analyses of rigid cuticles of the red flour beetle, *Tribolium castaneum*, have revealed not only numerous horizontal chitin-protein laminae but also vertically oriented columnar structures called pore canal fibers. This structural architecture together with tyrosine metabolism for cuticle tanning is likely to contribute to the rigidity and coloration of the beetle exoskeleton.

Addresses
[1] Department of Applied Biology, Chonnam National University, Gwangju 61186, Republic of Korea
[2] Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, United States

Corresponding author: Arakane, Yasuyuki (arakane@chonnam.ac.kr)

Current Opinion in Insect Science 2016, **17**:1–9

This review comes from a themed issue on **Molecular physiology**

Edited by **Takema Fukatsu** and **Ryo Futahashi**

For a complete overview see the Issue and the Editorial

Available online 12th May 2016

http://dx.doi.org/10.1016/j.cois.2016.05.004

2214-5745/

Introduction

Insects, belonging to the most diverse and successful animal phylum Arthropoda, have developed and acquired superior adaptability to the natural environment, excellent communication systems, and optimized body designs and functions throughout their evolution. In addition to these features, the exoskeleton or cuticle gives the insects excellent capabilities that help to account for their evolutionary success. The insect cuticle is a remarkable biomaterial/biomass primarily formed from structural cuticle proteins (CPs) and the linear polysaccharide, chitin. This strong extracellular material serves both as a skin and skeleton, protecting insects from environmental stresses and mechanical damage. The Coleoptera is the largest insect order, and its cuticle including that of the elytron (Box 1), which is a highly modified and tanned (sclerotized and pigmented) forewing, has been an attractive material for study not only because of its metallic and lustrous coloration but also due to its mechanical properties as a rigid and lightweight tissue. Here, we review the functional importance and the diversity of the genes involved in the tyrosine-derived cuticle tanning pathway, and the morphology and ultrastructure of rigid types of beetle cuticle. The functional importance, unique localization, and cross-linking of specific CPs in the formation of the exoskeleton are also discussed.

Beetle cuticle pigmentation

Several kinds of chemical pigments such as melanins, pterins, ommochromes, antraquinones, aphins, tertapyrroles, carotenoids and flavonoids/anthocyanins contribute to a variety of insect colors [1•]. However, there have been only a few studies focusing on the identification, biosynthesis, genetic regulation and function of these pigments in beetles. For example, carotenoid content is responsible for the variable orange-to-red coloration of elytra of the Asian ladybird beetle, *Harmonia axyridis*, and this red hue appears to be aposematically correlated with the content of their defensive alkaloid molecules [2]. Like other insect species, melanin-like and quinonoid pigments produced by tyrosine metabolism play a major role in the darkening of beetle cuticle [3,4,5•,6]. Tyrosine metabolism is also critical for cuticle hardening (sclerotization). Cuticle tanning involving tyrosine metabolism has been a study of major emphasis in our laboratories.

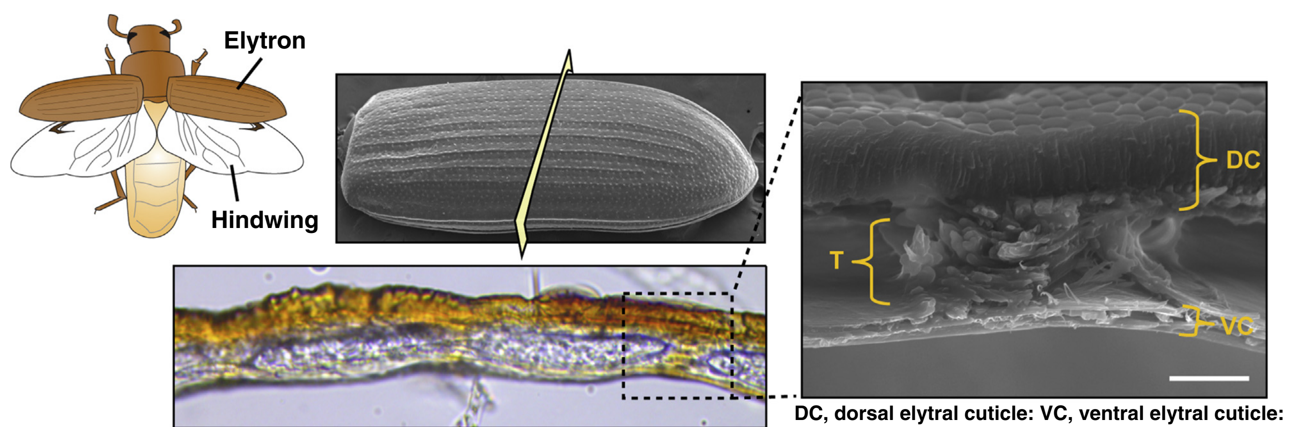
Tyrosine-mediated cuticle pigmentation and sclerotization

Cuticle tanning (pigmentation and sclerotization) is a complex process, which includes hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and decarboxylation of DOPA to dopamine (Box 2). Additional steps for melanization include oxidation of DOPA and dopamine to dopa-quinone and dopamine-quinone, conversion of these quinones to dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), via intramolecular cyclization, oxidation of DHI and DHICA to DHI-chrome and DHICA-chrome (melanochromes) and then polymerization of the melanochromes to form melanin-like pigments. Pigmentation involving *N*-acylated quinones requires *N*-acetylation of dopamine to

Box 1 The beetle elytron.

Primitive insects have two pairs of membranous flight wings, but during the evolution of the beetle lineage, the forewings lost their flight function and became modified as hard, rigid covers called 'elytra' [59–61], which is an example of appendage diversification of wings that have been diverted from their primary flight function and have evolved as a cuticular expansion [62]. This transformation is manifested by a greatly thickened and tanned elytral dorsal cuticle secreted by the forewing epidermis, and shares with the rigid body wall cuticle the same genetic cascades and tanning pathways in *T. castaneum* described above [6,47,63•].

The elytron is composed of dorsal and ventral layers of epidermal cells that secrete a thick upper and a thin lower cuticular lamination, respectively [21••,64,65]. The former is highly tanned and exhibits an ultrastructure very similar to that of other pigmented rigid cuticles in different body regions of adult beetles, whereas the latter is less pigmented and remains thin and membranous [21••]. In addition, there is a space between these two layers, which is filled with hemolymph and a large number of supporting pillar-like fibrous structures called 'trabeculae' that generally have a mechanical function separating and supporting the dorsal and ventral cuticular layers of the elytron [50,66].



DC, dorsal elytral cuticle: VC, ventral elytral cuticle: T, trabecula. Scale bar = 10 μm

Current Opinion in Insect Science

N-acetyldopamine (NADA) or *N*-β-alanylation to *N*-β-alanyldopamine (NBAD), oxidation of NADA and NBAD to NADA-quinone and NBAD-quinone and their polymerization to form the corresponding pigments. It should be noted that these quinone metabolites produced by laccase 2-mediated oxidation reactions also undergo cross-linking reactions with side chains of CPs such as histidyl residues for cuticle sclerotization [7]. *N*-acylation of the catecholamines reduces the rate of intramolecular cyclization of their quinone derivatives such that protein cross-linking reactions via the quinones become more prevalent.

Beetle cuticle structural coloration

Many beetle species including members of the families Scarabaeidae (scarab beetles) and Buprestidae (jewel beetles) have caught our attention because of their splendid metallic luster and iridescent body colors. Many of these beautiful colorations and some observed in several lepidopteran species [8–10] are actually 'structural colors' caused by a specific arrangement of cuticular structure(s) that differentially reflects light waves causing light interference. The structural coloration in beetles is mainly due to three iridescence mechanisms including multilayer reflectors, three-dimensional photonic crystals and diffraction gratings [11,12]. The multilayer reflector, which is the most common and extensively studied mechanism, is composed of alternating highly electron-dense and electron-lucent layers. Depending on the number of layers and also the thickness and distance between successive layers, different optical colors are produced by interference of light waves.

Recent studies have revealed that these reflectors can be found at different regions in beetle cuticles. For example, reflectors are found in the outer region of the cuticle (epicuticle and/or boundary between epicuticle and procuticle) of the jewel beetle, *Chrysochroa fulgidissima* [13], the aquatic leaf beetle, *Plateumaris sericea* [14] and the bronzed tiger beetle, *Cicindela repanda* [11], whereas they are localized in inner layers of the chitin-protein rich procuticle in many scarab beetles such as the rose chafer, *Cetonia aurata* [15]. Structural color, in addition, can sometimes be caused by a reversible change due to the environment. The yellow-greenish color of elytra of the Hercules beetle, *Dynastes hercules*, changes to black passively under high humidity conditions, and returns to a yellow-greenish color under dry conditions [16]. The yellow-greenish coloration is caused by a porous three-dimensional sponge-like structure located about 3 μm below the cuticular surface. The air in the holes of this structure are substituted for by water under high humidity, resulting in