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Characteristics of genes in carotenoid cocoon color, *Bombyx mori L.*

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Abstract

The cocoon's color of silkworm, *Bombyx mori L.* is usually white. But some are yellow, flesh and green colors because of modified characteristics. The yellow and flesh cocoons depend on carotenoid pigments, green cocoons are determined by flavonoid pigments. The cocoon's color is affected by the genes controlling penetration process from midgut to coelom and silk gland. *Y* (Yellow blood, 2-25.6) and *I* (Yellow-inhibitor, 9-16.2) genes are involved in the penetration process of carotenoid pigments from midgut to coelom, *C* (Outer-layer yellow cocoon, 12-7.2) and *F* (Flesh, 6-13.6) genes from coelom to silk gland. Therefore, the carotenoid cocoon's color depends on the genotype *Y, I, C* and *F* genes and their combination. Among them, *C* gene is sympathetic gene, which are known as *C, Cl* and *CD*. *C* (Outer-layer yellow cocoon) genes make yellow cocoons on outer-layer and white cocoons on inter-layer, and *Cl* (Inner-layer yellow cocoon) genes do yellow cocoons on inter-layer and dilute yellow cocoons on outer-layer. *CD* gene is known as making dilute yellow cocoons all layer. In this study, we have checked the dominance relation of *C* sympathetic genes among carotenoid genes for color cocoons by using strains related to the genes for color cocoons and investigated the aspect that pigments were penetrated in silk gland by action of each gene.

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Introduction

Carotenoid pigment is natural pigment being in plant, animal and microorganism. And this word has been originated in carotenoid which means the pigment of carrots. Carotene is yellow pigment and it has α-carotene, β-carotene and γ-carotene. Most of plants have β-carotene which prevents chlorophyll from oxidizing and attracts insects (Tsuchida *et al.,* 1998). Mulberry leaves that silkworm usually incepts have various pigments including carotenoid pigments. Looking at the character manifestation process of carotenoid genes for color cocoons which have been studied so far, carotenoid pigments in mulberry leaves that silkworms digested move from midgut to coelom by *Y* and $+^{I}$ gene, and then the carotenoid pigments circulate coelom as combining with lipophorin (Tsuchida *et al.,* 2004). The lipophorin combines to middle silk gland cell membrane by *C* (Outer-layer yellow cocoon) gene and *F* (Flesh) gene (Sakudoh *et*

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al., 2005). At that time, carotenoid pigments move into silk gland cell membrane (Tsuchida *et al.,* 2004).

C gene produces *Cameo2* and CBP protein which carries lipids, and this protein makes yellow cocoon at near the middle of middle silk gland mainly by penetrating lutein, on of carotenoid pigments (Chai *et al.*, 2014 ; Wang *et al*., 2014). *F* gene produces *SCRB15* protein which carries lipids, and this makes red cocoon at near the backward middle silk gland by penetrating β-carotene (Sakudoh *et al.,* 2010, 2013).

We checked the specific pattern to phenotype characteristics in the hybrid of carotenoid genes for color cocoons while breeding the strains having the genes for color cocoons. We makes an assumption that the pattern appears because of difference in expression caused by combination of *C* sympathetic genes. Also, we checked phenotype expression of characteristics through crossbreeding between *C* sympathetic genes.

Each of *C* sympathetic genes have different pigment penetration sites in the middle of middle silk gland and different colors. *C* gene gets involved in the pigment penetration process of the front of the middle of middle silk gland and makes yellow on outer-layer and white cocoons on inter-layer, C^{l} gene gets involved in the pigment penetration process of the back of the middle silk gland and makes yellow on inter-layer and dilute yellow cocoons on outer-layer, C^{D} gene gets involved in the pigment penetration process of the all of the middle silk gland and makes full-layer dilute yellow cocoons. Another carotenoid gene for color cocoons, *F* gene, makes the flesh color cocoon by involving in the pigments penetration process of backward middle silk gland (Nho, 1996).

In silk gland, carotenoid pigments start to move to the penetration sites for each gene when silkworms are 5 stars and 3 days. It is the easiest to observe silk gland on 5 stars and 4 days. After 5 stars and 6 days, its boundary is not clear and we have a difficulty to check the penetration sites for pigments because silk gland was fully dyed. But, we can check about the strongly dyed part.

We decided dominance relation between *C* sympathetic genes by checking the cocoon's color made through crossbreeding between *C* sympathetic genes. Also, we checked the penetration sites for carotenoid pigments from silk gland of strains having the carotenoid genes for color cocoons, and decided genotype for phenotype characteristics.

Materials and Methods

Silkworm strains

The 6 strains (207, 219, 301, 305, 306, 314) having color cocoon genes among the silkworm in insect genetic resources laboratory of National KyungPook University in Republic of Korea (Table 1).

Environment for feeding and method for breeding

All silkworm ate upper mulberry leaves, which took the sun over 12 hours and were provided with sunlight for 1 or 2 days. The temperature / moisture for feeding environment of silkworm were as in the following ; 28 ~ 29 °C / 80 ~ 90 % at 1, 2 stars, 27 ~ 28 °C / 70 ~ 80 % at 3 stars, 26 ~ 27 °C / 60 ~ 70 % at 4 stars, 25 ~ 26 °C / 50 ~ 60 % at 5 stars and silkworm were provided with lighting for 12 hours every-day. Method for breeding was that we sorted out 10 pupas from cocoons having the phenotype to match up with genotype. After they turned into moth, we made them breed and got the eggs, and then started feeding. The environment of hybrids was same as their parents.

The silk gland dissection

We chose randomly 50 individual of the pure breed and hybrid that are 5 stars and 5 days to experience and took pictures after dissecting them in 0.75 % NaCl or PBS buffer mixture.

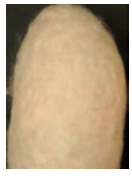
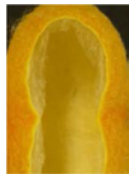
Sorting out phenotype of cocoons

After breeding, we used about 2,000 cocoons from each strain to experience, and classified them by phenotype characteristics of cocoons.

Results

We investigated the dominance relation between *C* sympathetic genes by breeding them after choosing the strains having *C* sympathetic genes among carotenoid genes for

Table 1. Phenotype and genotype of cocoon color by silkworm.

Genetic Character	Locus	Phenotypic Characteristics		Representative Strain	Origin
		Outer layer	Inner layer		
C (outer-layer yellow cocoon)	C (12-7.2)			a01	301
CD (Dilute-yellow cocoon)				ka47	219
CI (Inner-layer yellow cocoon)				s40V	306
F (Flesh)	F (6-13.6)			s40p, 4012	305, 314
CF (C + F)	C (12-7.2) F (6-13.6)				
CIF (CI + F)				c44	207

color cocoons of silkworm. Also, we checked the pigments penetration process in silk glands for each genes by dissecting the silk gland of the strains that have the carotenoid genes for color cocoons.

I. The result for the breeding experiment of C sympathetic genes

We sorted out the strains having $C^{I}F$, C^{p}, C and ^{+C}F gene to check the dominance relation between C sympathetic genes, we crossed $C^{I}F$ (207 strain) and ^{+C}F (305, 314 strain), C^{p} (219 strain) and C (301, 306 strain), and $C^{I}F$ (207 strain) and C^{p} (219 strain). We arranged the phenotype characteristics of the cocoons given after breeding, and then arranged dominance relation between each gene. Through this experience, we got three outcomes. outcome ① : We have gotten the cocoons having the phenotype of $C^{I}F$, C^{p} and CF gene by breeding $C^{I}F$ *and* ^{+C}F gene strains. Therefore, C^{I}, C^{p} and C gene are dominant to $^{+C}$ gene (Fig. 1). outcome ② : We have gotten the cocoons having the phenotype of C gene by breeding C^{p} and C gene strains (Fig. 2). outcome

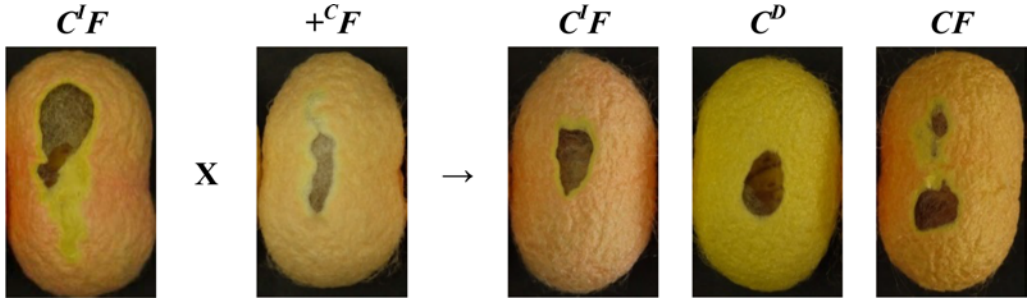


Fig. 1. 84 % of about 2,000 cocoons produced by breeding 207 (C^lF) and 305 ($+^cF$) had the phenotype characteristics same as C^lF gene, and 11 % had things same as C^D, and the rest of 5 % had *CF* gene.

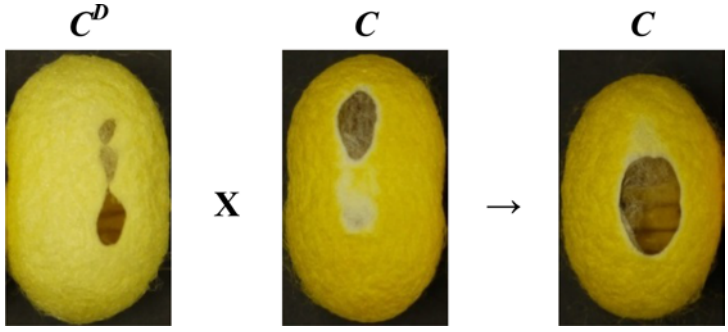


Fig. 2. All of 2,000 cocoons that we got by breeding 219 (C^D) and 301 (*C*) had phenotype characteristics same as *C* gene.

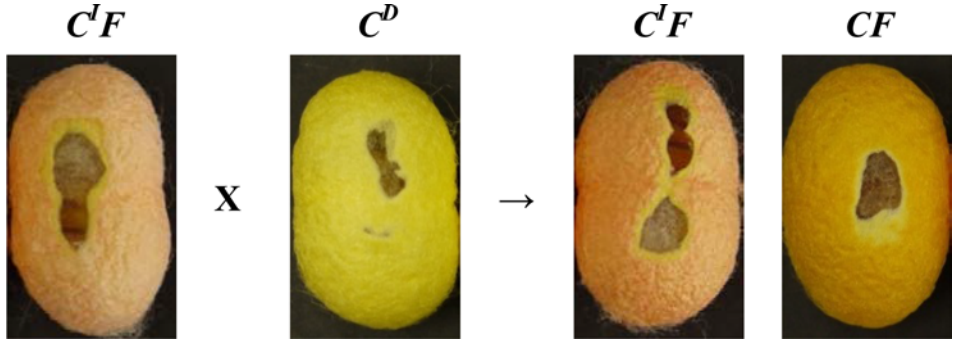


Fig. 3. 65 % of about 2,000 cocoons that we got by breeding 207 (C^lF) and 219 (C^D) had phenotype characteristics same as C^lF gene, and the rest of 35 % had same as *CF* gene.

③ : We have gotten the cocoons having the phenotype of C^lF and *CF* gene by breeding C^lF and C^D gene strains. Therefore, C^l gene is dominant to C^D gene (Fig. 3). Also, analyzing outcome ① and ③ could tell you that C^l gene is dominant or co-dominant to *C* gene.

As a result, the final dominance relation between *C* sympathetic genes is following ; $C^l \geq C > C^D > +^c$. Also, we checked that alo-dominant because it expressed independently unlike other *C* sympathetic genes.

II. The pigmentation of silk glands for each gene

C (Outer-layer yellow cocoon) gene and *F* (Flesh) gene are carotenoid genes for color cocoons, and it is said that *C* gene has 3 sympathetic genes until now. As I explained ahead at introduction, *C* sympathetic genes and *F* genes decide the color of cocoon by controling penetration process of carotenoid pigments at different positions in silk gland. Although having carotenoid genes for color cocoons, if silkwroms have not *Y*

Fig. 4. For C^lF gene. C^l gene expressed the pigmentation at back of middle of middle silk gland, and F gene at back of middle silk gland. It is said that C^p gene makes dilute yellow cocoons all-layer because pigments penetrate all of middle of middle silk gland. But we found out that C^p gene could make all-layer dilute yellow cocoon just by penetrating pigments at front of middle silk gland. *C* gene expressed the pigmentation at front of middle of middle silk gland, and $+^cF$ gene at back of middle silk gland

gene, they could not make the color cocoons because pigments can not be penetrated from midgut to coelom without *Y* gene. Therefore, the strains we experienced basically have *Y* gene. Just having *Y* gene, can cause pigmentation at silk gland cell (Kozo *et al*., 2004). We could not definitely distinguish the specific parts only from checking the pigmentation of silk gland cell. But we could check the parts that pigments penetration for each part in silk gland caused intensively on, also we checked the pigmentation of silk gland for each genes by using 5 stars 4 days silkworms(Fig. 4), which are useful for observing pigments penetration process.

Discussion

The layers of cocoons are divided into 3 parts, the inner, middle and outer layers. But, we named the middle layer as the inner layer. Because the inner layer is thin and easily separated like cocoon floss of outer layer. In general, it is said that if the outer layer of cocoons is yellow color, they are yellow cocoons even though not being entirely yellow color. But, the inner layer of the real yellow cocoons have various color such as yellow, citrine white and so on. To use the yellow cocoons as cloths without any dyeing processing, you have to breed the cocoons that have gold color for all layers. Therefore, we need to investigate genotype of various kind of yellow cocoons, that depend on phenotype, and analyse the association among them.

We chose and breeded the silkworms having carotenoid genes for color cocoons, and checked the expression patterns of cocoon's color. Using the patterns, we investigated the dominance relation among *C* sympathetic genes for yellow cocoons and *F* genes, and then analysed the association between color cocoon genes and pigments penetration in silk gland.

I. The dominance relation among carotenoid genes related to cocoon's color

While breeding the strain that have gold color for all layer, we found out that when the strain obtaining C^p gene and another strain obtaining *C* gene were breeded, they produced the cocoons that only have phenotype of *C* gene. Based on the result, we judged that it is possible for dominance relation to be caused among *C* sympathetic genes. As carotenoid genes related to cocoon's color, there are *C* (Outer-layer yellow cocoon) gene and *F* (Flesh) gene. *C* gene is the representative sympathetic genes of the genes for color cocoon, and *F* gene is essential to having characteristics for flesh cocoons.

As a result, the dominance relation of C sympathetic genes is $C^l \geq C > C^p > +^c$. We bred strains [C^lF (207), C^p (219), *C* (301, 306), ^{+C}F (305, 314)] having *C* sympathetic genes based on

Mendel's laws.

While experiencing to find out the dominance relation, we checked colors of about 2,000 cocoons for each strain eggs and separated them. As a result, we judge that genotype might be hetero-junction. That is because the cocoons from outcome ① and ③ have different characters that their parents do not have.

II. The pigments penetration site of each carotenoid gene for color cocoons

We conducted dissection experiment on the basis of pigments penetration sites in silk gland of carotenoid genes for color cocoons. As a result, it would seem that carotenoid pigments penetrated silk gland, but it had difficultly to distinguish the theoretical clear sites and we can just check the sites that pigments were intensively penetrated. The specific point is that a strain expressed phenotype of C^{p} gene by intensively penetrating in front of middle of middle silk gland unlike an existing theory about pigments penetration sites of C^{p} gene in middle silk gland. The other genes expressed the pigmentation same as an existing theory.

The mechanism about how carotenoid pigments move from silk gland cell to lumen has not yet been found out. We need directly to study about silk gland lumen because the cell-layer of silk gland is lightly colored with just yellow blood genes (Sakudoh *et al*., 2007).

To commercialize cocoons without any dyeing processing, we need the cocoons having pigments all-layers. For this, it should be the first to study about the expression of phenotype according to combination of carotenoid genes for color cocoons. Therefore, in this study, we verify that breeding color cocoons is possible by using dominance relation of *C* sympathetic genes without analysing genotype of carotenoid genes for color cocoons.

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