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Cocoon Spinning Behavior in the Silkworm, *Bombyx mori* : Comparison of Three Strains Constructing Different Cocoons in Shape

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ABSTRACT—The spinning behavior of the silkworm, *Bombyx mori*, was recorded on videotapes from two angles and analysed by three dimensional computer graphics using the Japanese (J. 124), Chinese (C.124) and their hybrid (J.124 × C.124) strains. These strains constructed typical peanut-shaped, spherical and ellipsoidal cocoons, respectively. Linear representation of the spinning posture revealed that larvae fixed the posterior half of the larval body (6th to 13th segment) and spun silk moving their anterior half (1st to 5th segment) for the most spinning period in all strains used. Little difference was observed in the average spinning speed among them. The Japanese strain spun primarily in a S-letter posture and changed its direction frequently. The larva of Chinese strain often assumed a C-letter posture and showed direction-changing behavior with comparatively lower frequency. The hybrid larva threw the head back largely in an U-letter shape during most of the spinning period and showed cocoon expansion behaviors most frequently. The cocoon expansion behavior occurred mainly at both ends of the peanut-shaped cocoon (J.124), at the center part of the spherical cocoon (C.124) and at both shoulders in the ellipsoidal cocoon of the hybrid strain. Thus, there exist strain-specific features in the spinning behavior, and it is suggested that the main behavioral factors affecting cocoon shape formation are the spinning posture and the cocoon expansion behavior during spinning.

INTRODUCTION

Experimental studies of the cocoon spinning behavior in insects were first done by Yagi (1926). This pioneering study was followed by Yanagisawa (1941, 1942a, b) and Yokoyama (1951) for the domesticated silkworm, *Bombyx mori*, Van Der Kloot and Williams (1953a, b) for the giant silkworm, *Hyalophora cecropia*, and Lounibos (1975, 1976) for the Chinese oak silkworm, *Antheraea pernyi*. These studies were based on experimental results obtained by visual observations, chemical or surgical treatments, kymograph tracing of the movement of spinning larvae, but nobody had tried three dimensional analysis of the cocoon spinning behavior until recently, probably mainly due to the difficulty of methodology for small animals.

Miura and his coworkers devised a simple method for measuring the cocoon spinning behavior of the silkworm, *B. mori*, using two video cameras and a personal computer (Miura *et al.*, 1991a). They have also developed a computer program named 3DASBS after accumulated statistical consideration

of the relationships between accuracy and measurement error, by which we can reconstruct the spinning behavior as three dimensional graphics on a display to analyse the successive process of larval body movement, spinning area, and spinning speed, etc. (Miura *et al.*, 1991b, 1993, 1994, 1995; Sugiura *et al.*, 1994). Using the system, they have clarified basic aspects of complicated spinning behaviors: characteristic behaviors of scaffold construction process for cocooning, stage-dependent changes of spinning region and spinning speed, periodical reverse changes of larval body direction etc. (Miura *et al.*, 1997, 1998). However, the most intriguing question on how cocoon shape is determined have not been analysed yet.

Larvae of the silkworm, *B. mori*, spin variously shaped cocoons which are typical of particular strains or races: some make globular cocoons, and others ellipsoidal or peanut-shaped cocoons. There also exist some mutant strains which make peculiar shaped cocoons, *i.e.*, attenuated at both ends or with a thin part in the center (Chikushi, 1972). Although these cocoon shapes were found difficult to be correlated to a limited number of genes (Doira, 1978), the differences in shape are considered to be attributed to the behavioral characteristics during the formation of the cocoon.

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In order to clarify the mechanism of cocoon shape formation, we tried in this study to analyse characteristic features of spinning behavior in three strains of the silkworm, *B. mori*, which make different shapes of cocoons, using the method established by Miura *et al.* (1991a, 1991b, 1993, 1994, 1995) and Sugiura *et al.* (1994).

MATERIALS AND METHODS

Experimental animals

Two parental strains of the domesticated silkworm, *B. mori*, (Japanese No.124 and Chinese No.124) and the hybrid strain (J.124 × C.124) were used as experimental animals. The Japanese strain

makes a peanut-shaped cocoon which has a narrow part in the center, the Chinese strain spins a globular or spherical cocoon, and the hybrid constructs rather an ellipsoidal one (Fig.1). The mean cocoon weights and cocoon shell percentages (cocoon shell weight / cocoon weight, × 100) were 1.33 g and 17.8% in the J.124, 1.57 g and 19.9% in the C.124, and 1.86 g and 21.4% in the hybrid strain. Thus, the hybrid larvae produce larger cocoons than the parent strains. Larvae were reared routinely at 25°C on fresh mulberry leaves. Developmental stages were determined by the method described by Kiguchi *et al.* (1985). To record the movement of each part of the larval body, a wandering larva was marked with red magic pen on the body at 15 points (p1 to p15) as shown in Fig. 2, where p1 and p13 represent the spinneret and the terminal segment, respectively. The two marks p14 and p15 were put only on the left spiracles to discriminate the direction of the movement.

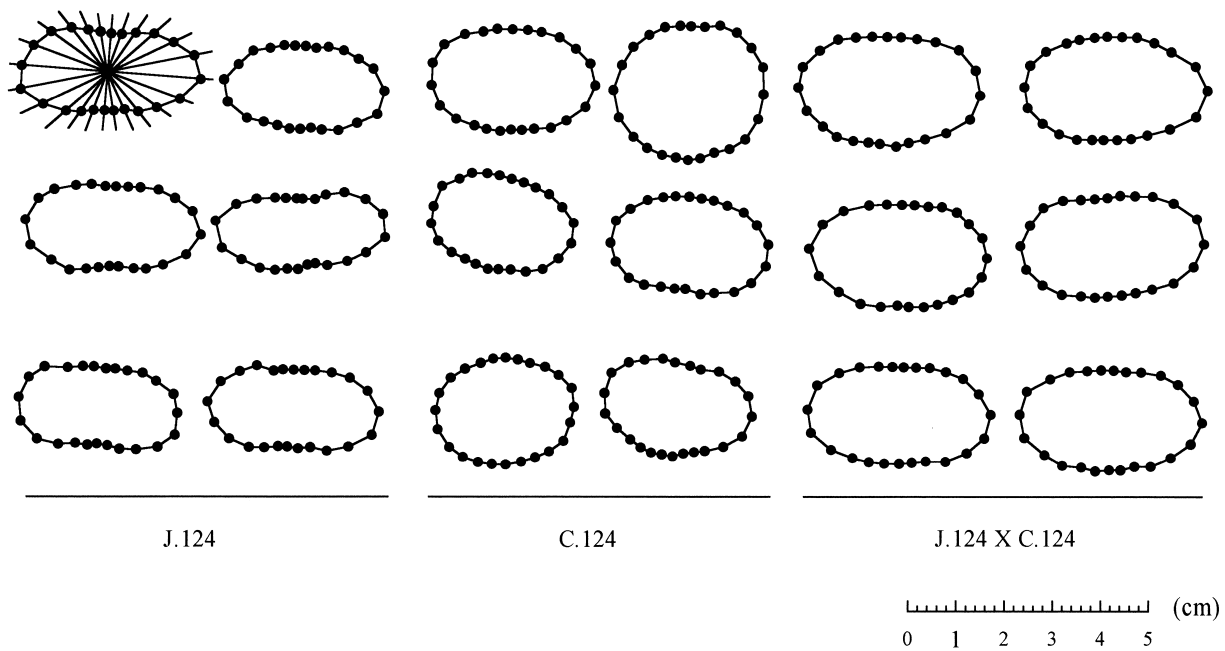


Fig. 1. Cocoon shapes of the silkworm strains used in this experiment. They are drawn on CRT based on the 24 intersecting points of cocoon outline and the lines from the center as seen at the upper left in the figure.

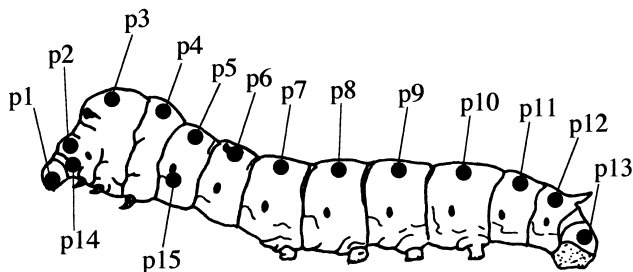


Fig. 2. Points marked on a wandering larva for measurement of the spinning behavior. p1: spinneret; p13: the terminal segment; p14 and p15: left 1st and 2nd spiracles marked for orientation of the larval body.

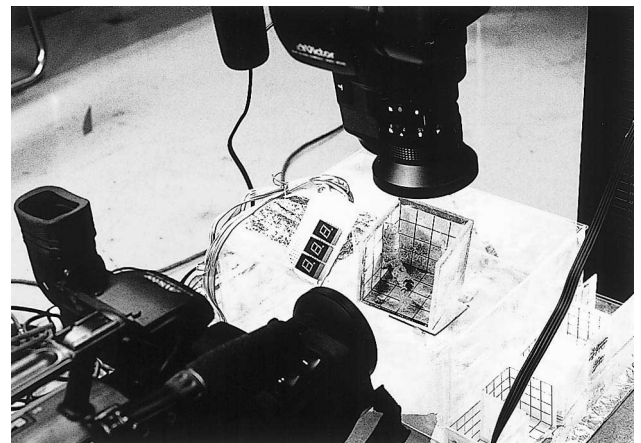


Fig. 3. Recording method by two video cameras. Inner surface of the acrylic box (40 × 40 × 40 mm) is lined with a graph paper for 3 dimensional measurement.

Recording on video tapes

According to the method described by Miura *et al.* (1991a), a wandering larva marked with a pen was put into an acrylic box of $40 \times 40 \times 40$ mm and the spinning behavior was recorded using two video cameras from two different angles, one was from the upper and the other from the lateral side (Fig. 3). A timer was also set to align the two videos taken at the same time by the two different video cameras. The temperature was maintained at $27 \pm 1^\circ\text{C}$ throughout the recording. Recording was continued until the marks became invisible due to visual obscurement by the silk thread of the cocoon.

Observation and analysis of the video tapes

First, visually distinguishable characteristics of the spinning behaviors, such as direction-turning behavior and cocoon expansion behavior which are mentioned later, were observed directly from the pictures displayed on the screen of cathode-ray tube (CRT). Then necessary site information of the marks put on the various parts of the larval body as primary pixel data was incorporated onto the computer disc by a mouse cursor. Two transformation matrices were determined using the relationship between known locations in the box and the positions on the CRT. A three-dimensional position of the

silkworm larva was reconstructed by the weighted least squares method using those transformation matrices from two values obtained on the CRT (Miura *et al.*, 1991a). A computer software 3DASBS (Miura *et al.*, 1991a ; Sugiura *et al.*, 1994) was used for this reconstruction. This was enable us numerical processing of the data to analyse the behavior from various angles.

RESULTS

In a previous paper, using the hybrid strain of the silkworm, *B. mori*, resulting from the cross between J.124 and C.124, we presented a developmental and behavioral timetable for the final 5th instar (Kiguchi *et al.*, 1985). The first visible sign of the initiation of the larval-pupal transformation is the pigmentation of the spinneret. This spinneret pigmentation occurs at about 6 hr after the larval body weight reaches its maximum. Then, the larva begins to wander about 8 hr after the beginning of pigmentation. When a wandering larva

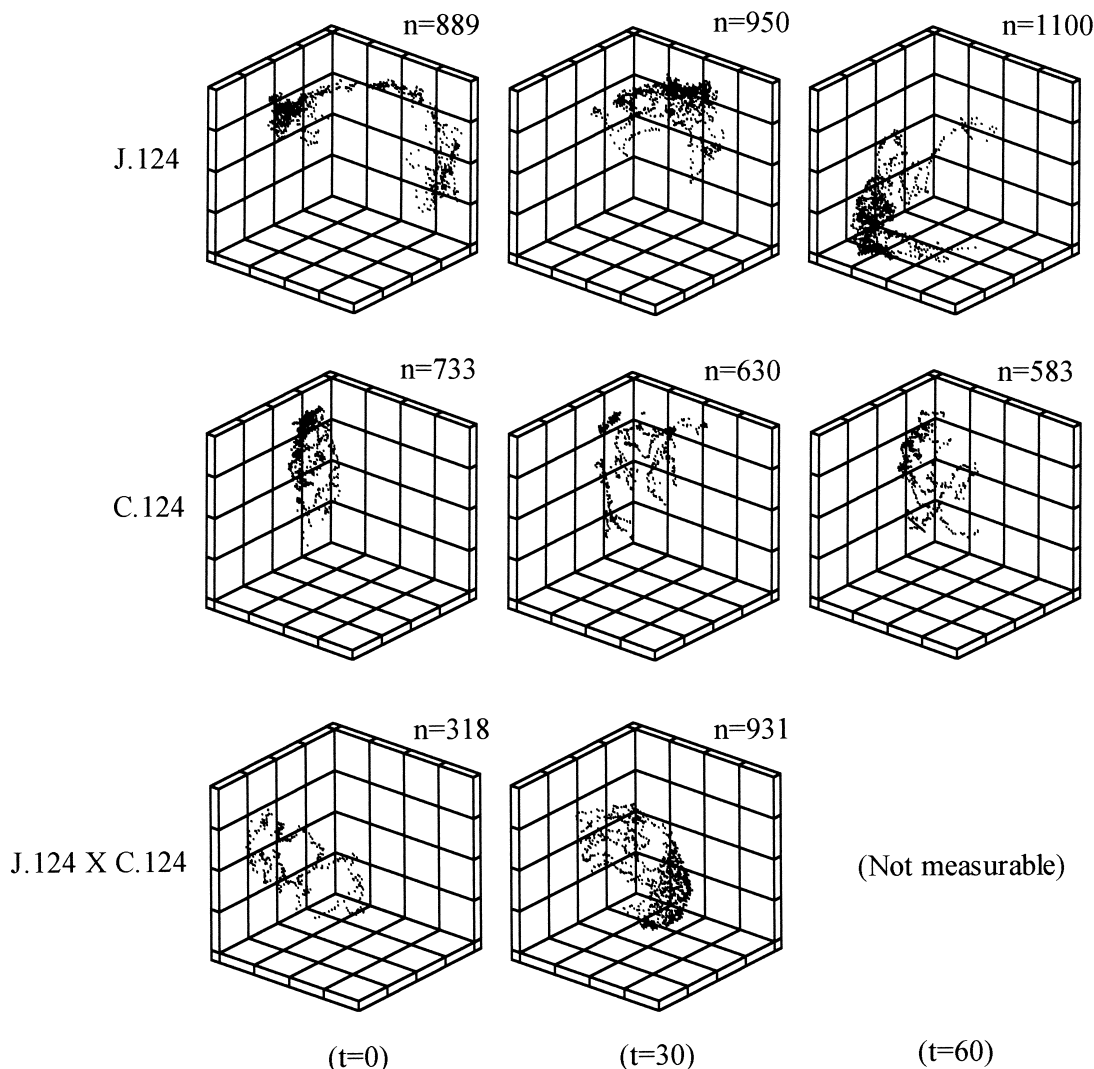


Fig. 4. Changes of spinning regions in the three strains. The spinning regions were expressed by the distribution of p1 position (spinneret, Fig. 2) measured at 0.1 sec intervals for 2 min; just after GP ($t = 0$), 30 min ($t = 30$) and 60 min ($t = 60$) after GP. n = the number of samples defined from two angles.

is kept under a favorable condition for cocooning, it first makes a scaffold for the cocoon by spinning small amount of silk, then purges its gut and begins to spin the cocoon. Since the gut purge (GP) behavior is very distinct and physiologically important, we adopted the behavior as a starting time for analyses of behaviors.

Spinning region

Generally silkworm larvae first spin a wide scaffold of silk, then gradually reduce their spinning region to the final cocoon size (Miura *et al.*, 1997). In most hybrid strains which usually spin more silk than the parental strains, GP occurs during the time when the larvae are spinning the thin outer cocoon layer a few hours after the completion of the scaffold (Kiguchi *et al.*, 1985). To examine the change in the spinning region, the position of the spinneret (p1 in Fig. 2) was examined every 0.1 sec for 2 min at three different stages: 0 (immediately), 30 and 60 minutes after GP. The distribution of the position is shown in Fig. 4 for the three silkworm strains used. In the hybrid strain, the spinneret position was confined

within the final cocoon size at GP (Fig. 4a), indicating that the larva has finished construction of the scaffold before GP. Observation of the spinneret position became impossible one hour after GP because of obscurement by the silk thread spun. By contrast, in both parental strains, the spinneret positions were found in a broader area 60 min after GP, indicating that they had not yet finished the scaffold construction (Fig. 4b,c). Thus, the timing of GP relative to scaffold formation differs in the hybrid strain.

Larval body posture during the spinning

Once silkworm larvae begin spinning, they continue without resting for more than 2 days. During this time they repeat the fixation and movement of the posterior half of the larval segments with abdominal and caudal legs. Fig. 5 shows a linear representation of the spinning posture during the fixation about 30 min after GP which was reconstructed from the movement of 13 points (p1 to p13) marked on the experimental animal (Fig. 2), as measured at 1 sec intervals for 20 sec. The larvae fixed the posterior half of the larval body (6th to

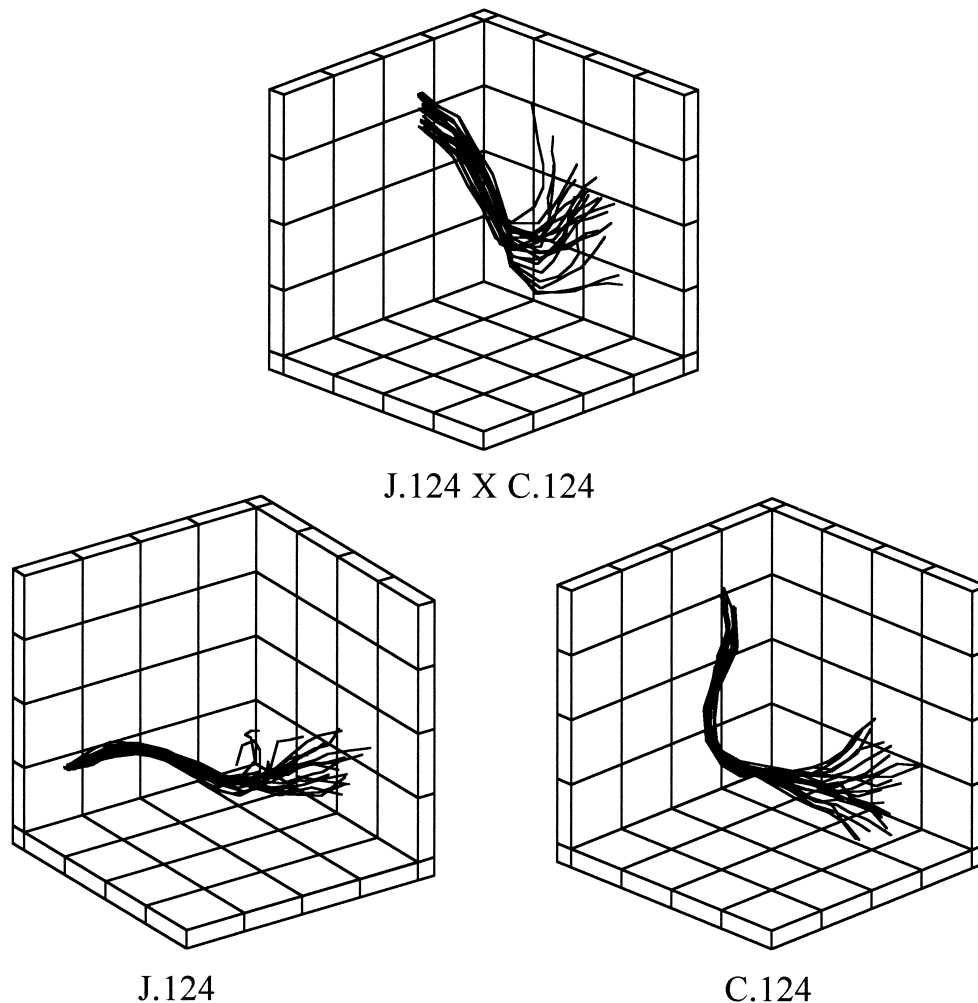


Fig. 5. Linear representation of the spinning postures in the three strains. The movement of the marks from p1 to p13 (Fig. 2) was measured at 1 sec intervals for 20 sec at 30 min after GP.

13th segment) and spun silk moving the anterior half from the head to the 5th larval segment in all strains used. There were strain-specific features in such spinning postures: the J.124 larva twists its body into a S-letter form, the C.124 larva never twists but bends its body into a C-letter form, and the hybrid strain throws back its head largely in an U-letter form (Fig. 5).

Based on the data shown in Fig. 5, we calculated the mean spinning postures by 3DASBS (Sugiura *et al.*, 1994), and obtained the mean bending degree of the spinning posture (Fig. 6). The mean degree (θ in Fig. 6) was about 115° in the hybrid strain, and 73° and 85° in the Japanese and Chinese strains respectively. Thus, it was larger in the hybrid strain than those of the parental strains. According to our observation, these behavioral characteristics in the spinning posture seemed to be obvious throughout the latter half of the scaffold construction and early cocooning stages (data not shown).

Direction-changing behavior and cocoon-expansion behaviors

The frequencies of two conspicuous behaviors, direction-changing behavior and cocoon-expansion behavior, were examined on the CRT screen. During the spinning period, larvae often turn direction to change the spinning region, and also frequently exhibit the cocoon expansion behavior so that they can make symmetrical cocoons both in shape and thickness. Observation was done from the time of GP until the time of visual obscurement by the silk thread spun, which was longer for two parental strains (450 min for J.124 and 500 min for C.124) than for the hybrid (300 min). The time and the duration of each direction-change are shown in Fig. 7. The changing frequency was higher in the Japanese strain which makes peanut-shaped cocoons than in the Chinese strain which constructs spherical cocoons (16.0 vs 9.6 times/h) (Table 1). The mean duration required for one moving behav-

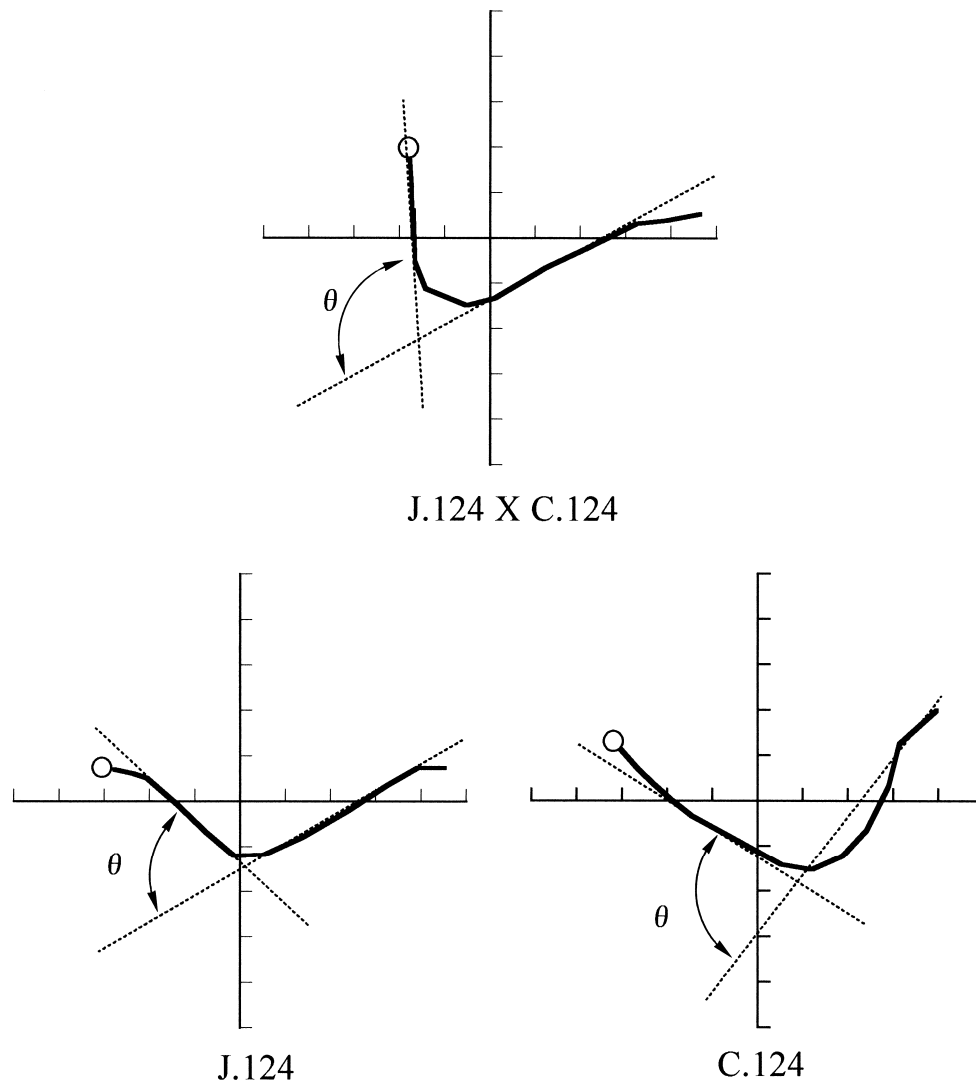


Fig. 6. Comparison of the mean spinning postures among the three strains. The mean larval posture was calculated for each strain based on the data shown in Fig. 5. Circles in the figure mean the head part of the spinning larva. Note the larval bending degree (θ) of the hybrid strain is larger than those of the parental strains.

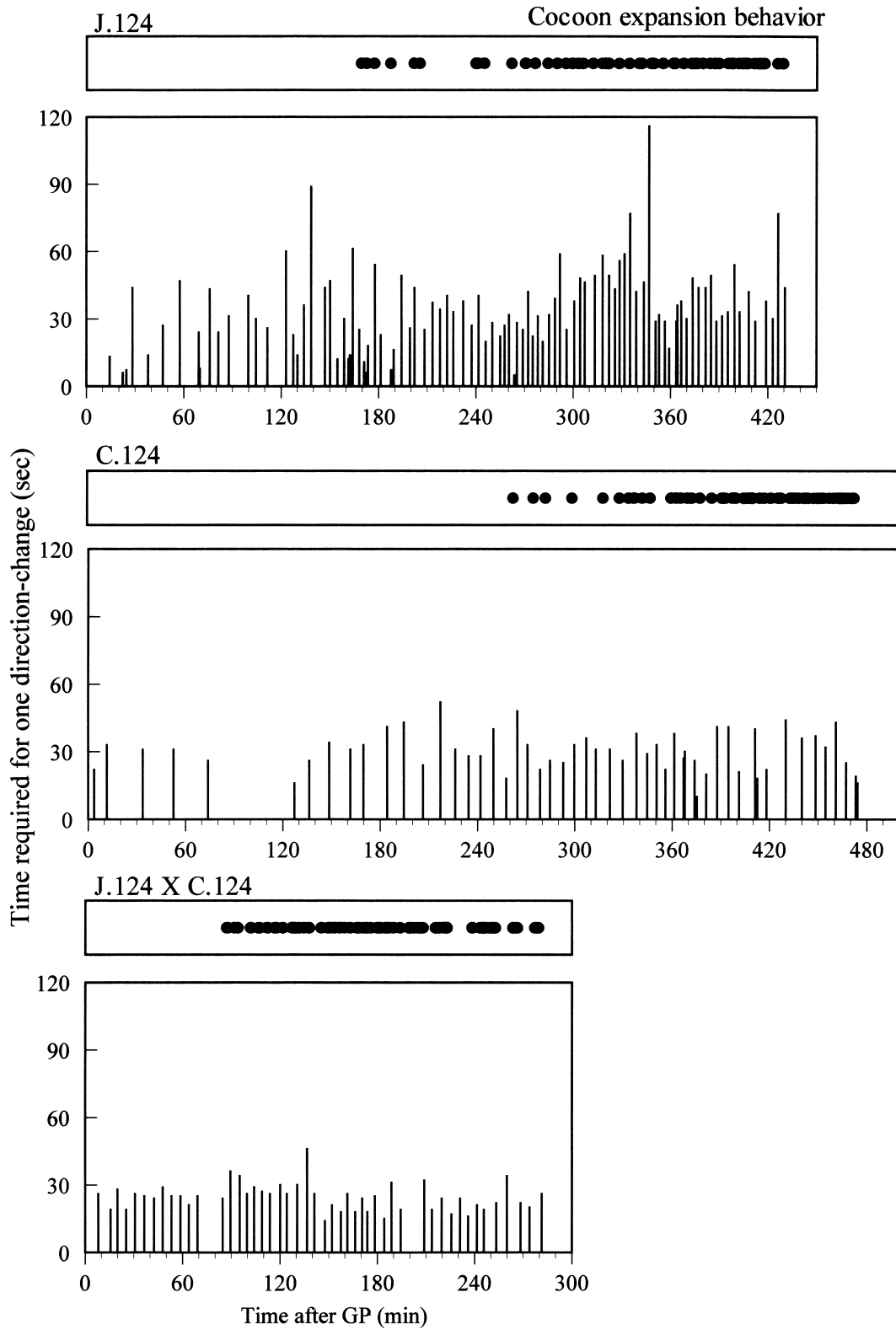


Fig. 7. The frequencies of direction-changing and cocoon expansion behaviors during the spinning period. The frequencies were carefully examined by direct observation of videotapes from GP until the time of visual obscurement by the silk thread spun. Vertical line indicates the time (sec) required for each direction change. The time showing cocoon expansion behavior was denoted as dots in the upper box.

Table 1. Comparison of characteristics of the spinning behaviors among the three strains used.

Name of strain	J.124	C.124	J.124 × C.124
Cocoon shape	Peanut shape	Spherical shape	Ellipsodal shape
Average spinning speed Mean ± S.D. (mm/sec)	9.6 ± 2.1 (n = 85)*	9.4 ± 1.8 (n = 82)*	9.4 ± 1.4 (n = 84)*
Spinning posture	S-letter form	C-letter form	U-letter form
Bending degree in the mean spinning posture (θ)	73°	85°	115°
Frequency of direction-change behavior (time/h)	16.0	9.6	11.7
Time required for one direction- change Mean ± S.D. (sec/one time)	35.0 ± 18.0 (n = 98)*	30.7 ± 8.8 (n = 53)*	24.5 ± 6.0** (n = 48)*
Frequency of cocoon expansion behavior*** (time/h)	10.0	14.0	24.2
Major site showing cocoon expansion behavior	Both ends	Center	Both shoulders

* "n" in the parenthesis means the number of samples measured.

** Significant at 5% level (t-test) with J.124 and C.124.

*** The frequency was measured during 3 hr after the first cocoon expansion behavior was observed.

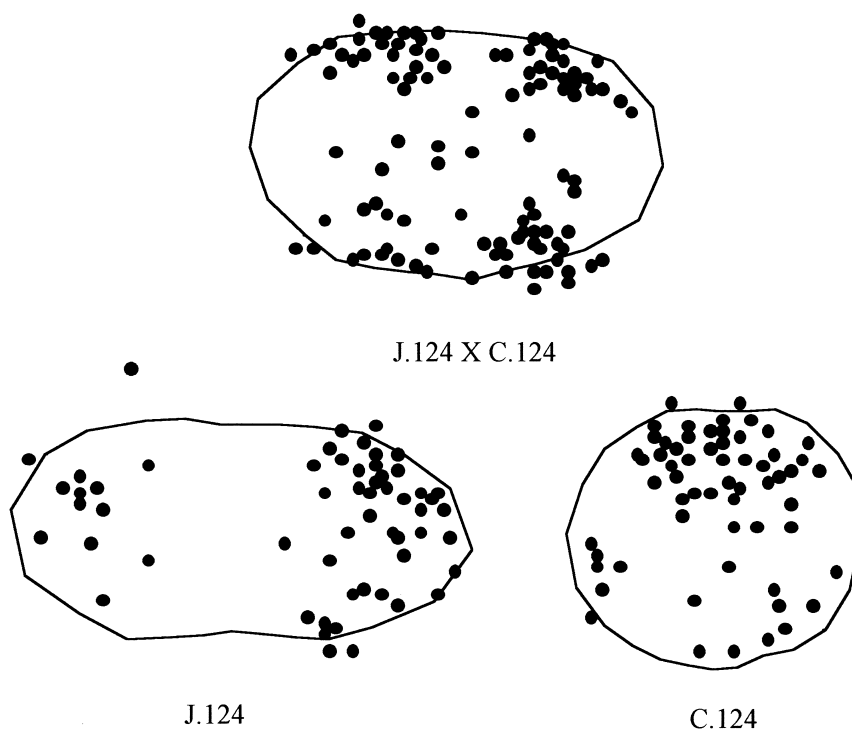


Fig. 8. Comparison of the sites showing cocoon expansion behavior among the three strains. First, the outline of cocoon shape was drawn on CRT, then the sites of cocoon expansion behavior were identified by direct observation of videotapes from GP until the time of visual obscurement by the silk thread of the cocoon. Each dot indicates the site where a spinning larva pushes the cocoon layer outward for a moment to expand the cocoon.

ior was 35.0 sec in the Japanese strain and 30.7 sec in the Chinese strain (not significant at 5% level), but it was 24.5 sec in the hybrid strain (significant at 5% level when compared with parental strains). Thus, the direction-changing behavior in the hybrid seemed more smooth and rhythmical than those of the parent strains (Fig. 7).

The cocoon expansion behavior is an action whereby a larva stretches its neck region and press the head outward for a moment to expand the cocoon. The expanded distance is usually 2.8 to 3.3 mm (Miura *et al.*, 1997). The times when such behavior was observed are also shown in Fig. 7. This behavior first occurred about 80 min after GP in the hybrid, whereas it began much later in the Japanese and Chinese strains, about 170 and 260 min after GP, respectively. As seen in Fig. 8, the cocoon expansion behavior in the Japanese strain occurred mainly at both ends of the peanut-shaped cocoon, whereas it occurred more often at the center part of the cocoon in the Chinese strain, and at both shoulders in the hybrid strain.

Spinning speed

By 3-D computer graphic analysis of the spinneret movement, spinning speed can be estimated with sufficient statistical precision (Miura *et al.*, 1993). Since the spinning speed changes rapidly for a short time and also changes significantly depending on the process of spinning (Miura *et al.*, 1993), it was estimated as an average speed based on randomly selected data taken during the total period of observation. Unclear data in dead angles were omitted from the calculation. As shown in Table 1, the average spinning speed was similar and there was no significant difference among the three strains, ranging from 9.4 to 9.6 mm/sec, despite the large differences in their cocoon shape and the amount of silk spun.

DISCUSSION

The analysis system (3DASBS) developed by Miura *et al.* (1991a) and Sugiura *et al.* (1994) made it possible to represent the spinning behavior as 3-D graphics on CRT and to treat the behavioral parameters statistically. In our experiments we took videotapes on several individuals for each strain and selected the most suitable one for the 3-D graphic analysis judging from the final cocoon size and shape characteristic of each strain. Moreover, our observations and conclusions were confirmed by partial analyses of the remainder when necessary.

The results obtained are summarized in the Table 1. The larval body shape (spinning posture) changed every few seconds from the beginning to the end of spinning. For most of the spinning period, the larvae often repeated the fixation and movement of the posterior half of the larval body (6th to caudal segment), so that they could make symmetrical cocoons. However, comparison of the main behavioral characteristics of these three strains showed significant differences in the spinning behavior. The larvae of the J.124 strain which made peanut-shaped cocoons spun in a S-letter posture when fix-

ing the posterior half of the larval body, and changed their spinning direction most frequently. This rapid changing may be due to the narrow and limited space in the cocoon. The Chinese larvae of the C.124 strain which produced spherical cocoons spun mostly in a C-letter posture, and their spinning was characterized by a much lower frequency of direction-changing behavior. By contrast, larvae of the hybrid strain which made large ellipsoidal cocoons, threw their heads and thoracic segments back well into an U-letter form during spinning. The direction-changing behavior in a cocoon seemed rhythmical. The hybrid strain was also characterized by a high frequency of cocoon expansion behavior when compared with the parental strains. From these observations, most probable factors relating to the cocoon shape formation seemed to be the larval posture and cocoon expansion behavior during spinning.

The findings on the relationship between spinning posture and the final cocoon shape are similar to those noted by Yanagisawa (1942a, b). Cocoon shape is also related to both the length and the girth of the larval body with a coefficient of correlation between body width/length and cocoon width/length of 0.79 ± 0.11 (Yokoyama, 1951). We also observed similar tendencies: short larvae make globular cocoons (data not shown). Possibly the body shape of the larvae influences the spinning posture.

Miura *et al.* (1997) suggested that body stretching (cocoon expansion) behavior is a factor responsible for the appearance of constricted parts in the center of a peanut shaped cocoon, because the behavior is more often observed in the species producing cocoons with remarkably constricted parts. In the present study, cocoon expansion behavior began relatively early in the hybrid strain which formed the ellipsoidal shaped cocoon and which began spinning the cocoon very early. However, this behavior was also seen in both parental strains, but significantly late and at different points corresponding to the different shapes of the cocoons. The reasons for the differences in this behavior which leads to differences in cocoon shape are as yet unknown.

As is evident from the Table 1, there exist strain-specific features in the spinning behavior. It is also interesting from a point of view of behavioral genetics to compare the behavioral differences among parent strains and the hybrid. In addition, there are many geographical and mutant strains in the domesticated silkworm, *B. mori*, including variants of cocoon shape and texture (Chikushi, 1972; Doira, 1978). These may provide good materials for the analysis of mechanism controlling spinning and cocoon shape formation. Studies are under way by combining to use such experimental strains and our analytical system of spinning behavior (Miura *et al.*, 1991a; Sugiura *et al.*, 1994).

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