Journal of Insect Biotechnology and Sericology 82, 00-00 (2013)

Shape of Silkworm cocoon changes with size in some races

Takahiko Nishioka[1*], Keisuke Mase[2], Zenta Kajiura[3], Mika Morishima[1], Takashi Kudoh[4]

[1] *Department of Textile System Engineering, Faculty of Textile Science & Technology, Shinshu University, 3-15-1, Tokida, Ueda 386-8567, Japan,*
[2] *Nihon University, College of Humanities and Sciences Department of General Studies, 3-25-40, Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan,*
[3] *Department of Applied Biology, Faculty of Textile Science & Technology, Shinshu University, 3-15-1, Tokida, Ueda 386-8567, Japan,*
Hitachi Plant Technologies, Ltd. 603, Kandatsu-machi, Tsuchiura Ibaraki, 300-0013, Japan

(Received ★★★★★, ★★★★; Accepted ★★★★★, ★★★★)

In general, the basic shape of Silkworm (*Bombyx mori*) cocoons is thought to depend upon race and not the size of the cocoon. That is, it is thought that within species, changes in the size of cocoons are proportional. The ratio of cocoon length to width is commonly used to numerically express the shape of cocoons. In the present study, we employed a Fourier coefficient in order to express the cocoon shape in detail. We found that in some Chinese silkworm races, the basic shape of the cocoon changed with size. The shapes of the geographical silkworm races have been precisely reported by Enokijima *et al*. through the ratio of the length to the width of the cocoon.

Key words: cocoon shape, cocoon size, Fourier coefficient, regression analysis

INTRODUCTION

The author et al. been investigating whether changes in the breeding environment of silkworms (*Bombyx mori*) affect the shape of the cocoons. The shape of cocoons does not always conform to the basic cocoon shape that is associated with a given silkworm race. Enokijima et al. (1985) described cocoon shapes for 268 geographically classified races. The report included the length, width, volume and the ratio of the length to the width for cocoons from each race. However, the report did not indicate why the length-to-width ratio was significantly correlated to the cocoon volume only for the Chinese race. We attempted to describe characteristics of the cocoon shape using Fourier coefficients from measured images of cocoons. When Fourier coefficients were used to describe the cocoon shape, it became apparent that the cocoon shapes of most silkworm races were distributed within a certain range, with the exception of some Chinese races.

We attempted to express the reason for the wide range of cocoon shapes observed for some Chinese races using the $a_0/2$ and a_2 Fourier coefficients.

MATERIALS AND METHODS

1. Cocoon shape measurement

We reared silkworm larvae on mulberry leaves donated by Laboratory of Agricultural Science, School of Textile Science and Technology, Shinshu University. Larvae were kept at 25°C ± 2°C and cocooned in 5 cm^3 cardboard boxes.

We examined the shape of the cocoons spun by the silkworm larvae and analyzed the ways in which the basic shape of the cocoons varied with cocoon size using the coefficients of a Fourier expansion. The basic shape of the cocoon was defined by a constant and two or three cosine terms of the Fourier series, as previously reported (Nishioka et al., 2001).

Because the Fourier series can contain an arbitrary number of terms, the shape of cocoons can be represented in more detail when the Fourier series is used than when the ratio of the cocoon length to cocoon width is used.

To obtain the cocoon shape, we measured the length in millimeters from the center to 128 points along the outer contour of each cocoon (Fig. 1). These measurements were plotted in a Cartesian coordinate system, as indicated by the wide line in Fig. 2. The thin line shown in Fig. 2 is the cocoon shape calculated from the mean term $a_0/2$

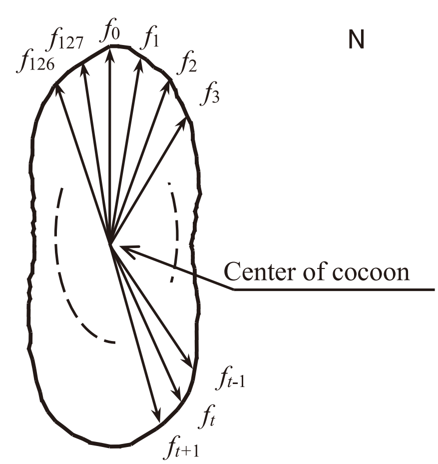

Fig. 1. Measurement method for *Bombyx mori* cocoons. The distance from the center of the cocoon to 128 separate points along the cocoon contour was measured in millimeters.

*To whom correspondence should be addressed.
Tel: +81-268-21-5381.
Email: tntecha@shinshu-u.ac.jp

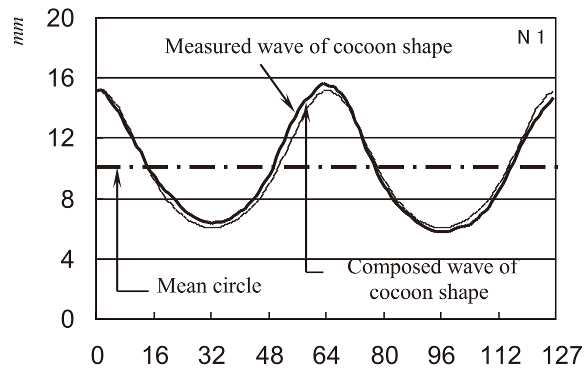

Fig. 2. The waves representing the shape of *Bombyx mori* cocoons. The wide line indicates the measured cocoon shape while the thin line indicates the shape calculated from Fourier coefficients $a_0/2$, a_2, a_4, and a_6. The mean circle refers to the mean distance from the center of the cocoon.

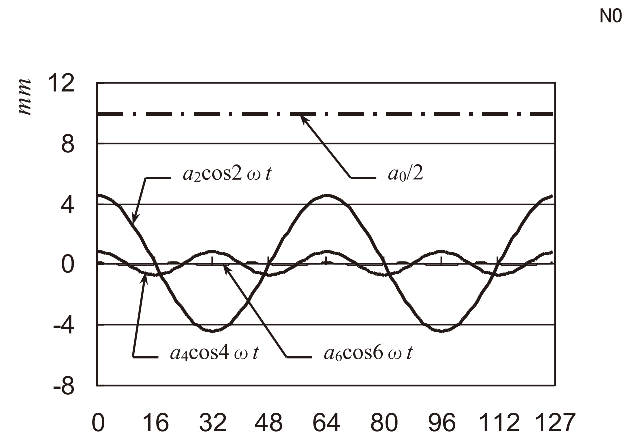

Fig. 3. The three significant waves and a constant term used to describe the *Bombyx mori* cocoon shape. $a_0/2$ is the average length (the mean circle) from the center to the contour of the cocoon and $a_k\cos k\omega t$ represents the k times cosine terms against the fundamental period.

and cosine terms a_2, a_4, and a_6. In this figure, the alternating long-and-short dashed line indicates the average distance from the center to the contour of the cocoon (mean circle). Figure 3 shows the individual waves that compose the cocoon shape. Of course, if all of the obtained coefficients were included in the calculation, the calculated wave might completely correspond to the measured cocoon shape.

2. Analysis

As can be seen in figs 2 and 3, the a_2 Fourier coefficient indicates the basic shape of the cocoon. That is, the shape of the cocoon is characterized mainly by coefficient a_2. We previously reported that there was a high correlation between the cocoon volume and the $a_0/2$ coefficient (Nishioka et al., 1998). Therefore, we examined the relationship between the $a_0/2$ and a_2 Fourier coefficients.

If the basic shape of the cocoon is similar, regardless of cocoon size, then the length and width should remain proportional over a range of cocoon sizes. That is, each silkworm race would have an inherent ratio of cocoon length to width. If this is true, the cocoon length-to-width ratio would not be significantly correlated with the cocoon volume.

The elements used to describe the basic shape of cocoons are included in a Fourier series, as shown in the following expression.

$$f(t) = \frac{a_0}{2} + \sum_{k=1}^{63} (a_k \cos k\omega t + b_k \sin k\omega t) \qquad (1)$$

If, for example, the size of Cocoon *A* were 80% of the size of Cocoon *B*, but both had the same shape, multiplying each term on right hand side of the equation for Cocoon *B* by the constant 0.8 would provide the term of the equation for Cocoon *A* (1). Similarly, to obtain the terms for a cocoon that is 120% the size of Cocoon *B*, each term in the expression for Cocoon *B* would be multiplied by 1.2 (1). Thus, in cocoons that are of different sizes but of the same shape, the ratio of any two arbitrarily chosen coefficients that are included in expression (1) remains constant. To examine whether the shape of cocoons changed with size, we performed a regression analysis of coefficient a_2 against coefficient $a_0/2$. If the basic shape of the cocoon changes to similar figures by varying in size, a restricted regression line that passes through the origin may be obtained. We used the following restricted regression model,

$$y = cx + \varepsilon_i \qquad (2)$$

where c is the ratio of Fourier coefficient a_2 against $a_0/2$. This variable also represents the slope of the regression line. The estimate of coefficient c was obtained using the standard least square method through the following formula:

$$\hat{c} = \sum_i x_i y_i \Big/ \sum_i x_i^2.$$

This regression model has no constant term and can be used to test the hypothesis that the regression line passes through the origin. Although this model does not attain the minimum variance assumed by mathematical statistics, this coefficient can be compared to the coefficient obtained by usual regression.

RESULTS

1. Japanese silkworm cocoon

Figure 4 shows a plot of the a_2 against the $a_0/2$ for race N01. Here, the coefficient $a_0/2$ is a constant term of a Fourier series that has been previously called the mean circle of the cocoon. In this figure, the solid line shows

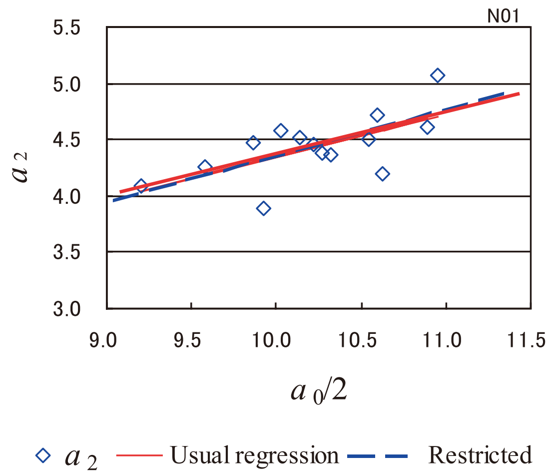

Fig. 4. Regression lines of a_2 against $a_0/2$ for race N01.

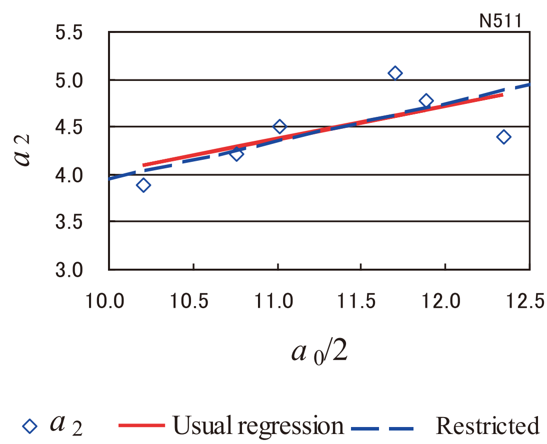

Fig. 5. Regression lines of a_2 against $a_0/2$ for race N511.

the usual linear regression line, and the broken line shows the restricted regression line. These two lines have nearly equal characteristics, indicating that the basic shape of cocoon did not vary with cocoon size. Figure 5 shows a similar plot for race N511. This figure indicates that race N511 had the same characteristics as race N01. Although the restricted regression line of race N603 shown in Figure 6 had a different slope than the usual regression line, the hypothesis that the regression line passes through the origin was not rejected. Table 1 shows the characteristics of nine races of the so-called Japanese silkworm cocoons that were used in this experiment, including intercepts of the regression equations. Zero is included in the 95% confidence interval for the estimated intercept value of all Japanese silkworm cocoons. Therefore, the hypothesis that the regression line passes through the origin could not be rejected. In other words, the basic shapes of all Japanese silkworm cocoons may be similar for all cocoon sizes.

2. Chinese silkworm cocoon

Figure 7 shows a plot of the a_2 against $a_0/2$ for race C505. This plot is similar to those for Japanese races, and

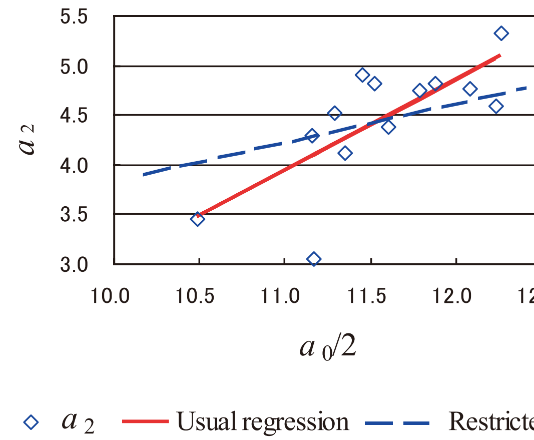

Fig. 6. Regression lines of a_2 against $a_0/2$ for race N603.

Table 1. Intercepts obtained from general regression and its upper and lower limits in the Japanese *Bombyx mori* cocoons

		95% confidence intervals		
races	n	intercepts	lower limits	upper limits
N01	14	0.5885	−2.2429	3.4198
N124	20	−0.8221	−1.8320	0.1879
N137	16	0.7983	−1.1035	2.7001
N511	6	0.5451	−5.5347	6.6250
N512	9	−0.5686	−2.8074	1.6703
N603	13	−6.0836	−12.4501	0.2830
SAWA-J	8	3.3977	−0.2737	7.0691
TN26	15	−0.5981	−2.6150	1.4189
TN38	15	0.4775	−5.1192	6.0741

indicates that the cocoon shape did not vary with cocoon size. Figure 8 shows a similar plot for race C514. In this figure, the regression line is different from those of the Japanese races. Similarly, Fig. 9 indicates that the regression lines for C108 (New) share the same characteristics as those for race C514. This means that, unlike the cocoons of Japanese races, the cocoon shapes of these other races varied with size. The intercepts of the regressions for Chinese silkworm races can be found in Table 2. Zero was not included in the 95% confidence interval for the intercept of regression lines for races C108 and C514. That is, the hypothesis that the regression line passes through the origin was rejected. The measured cocoon shapes of C514 and N01 are provided in Fig. 10 for comparison. This figure indicates that the size of race C514 cocoons depends primarily on their width (Fig. 10a). Thus, the basic shape of cocoons in this race changed with cocoon size because cocoon size variation was mainly due to cocoon width. The basic shape of race N01 cocoons (Fig. 10b) did not change with cocoon size because the ratio of the cocoon's length to width was similar for all cocoon sizes. A significant regression was not found, though an analogous characteristic of races C514 and C108 appears in C149. Some Chinese silkworm cocoons had large positive correlation coefficients, while others

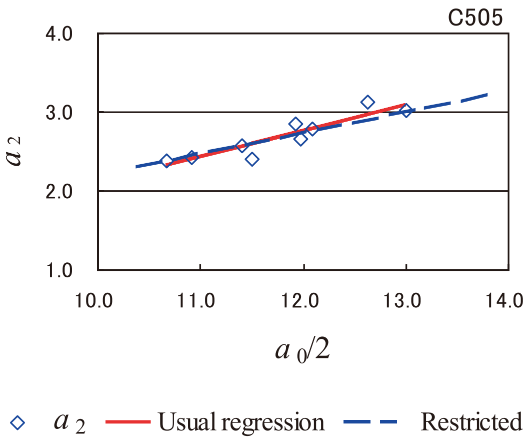

Fig. 7. Regression lines of a_2 against $a_0/2$ for race C505.

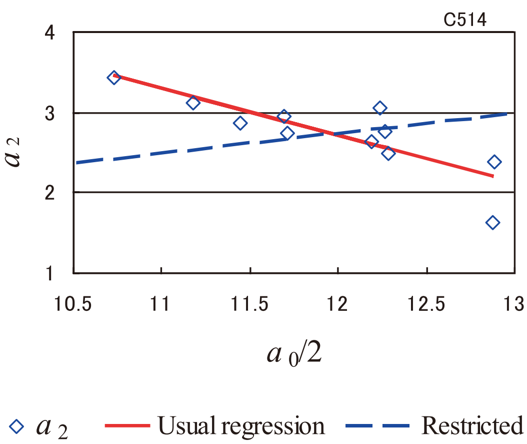

Fig. 8. Regression lines of a_2 against $a_0/2$ for race C514.

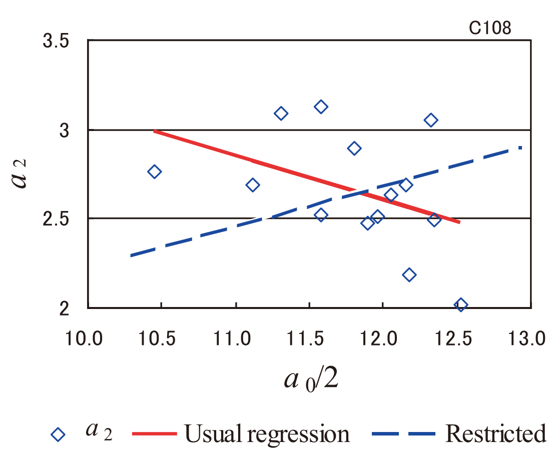

Fig. 9. Regression lines of a_2 against $a_0/2$ for race C108.

had large negative correlation coefficients. Positive correlation coefficients imply that the slope of the regression is positive, and cocoon shapes did not vary with size, as the Japanese silkworm cocoons. Negative correlation coefficients imply that the slope of the regression is negative, and the basic cocoon shape varied with size. It should be noted that not all Chinese races have cocoons that vary with size. as C514 and C108 do.

Table 2. Intercepts obtained from general regression and its upper and lower limits in the Chinese *Bombyx mori* cocoons

		95% confidence intervals		
races	n	intercepts	lower limits	upper limits
C108	14	5.5609	1.6832	9.4386
C149	14	4.1462	−0.4766	8.7691
C505	9	−1.2055	−2.7372	0.3261
C512	7	1.2170	−2.1915	4.6255
C513	7	−3.1635	−8.9521	2.6251
C514	11	9.6701	6.0643	13.2760
PCG	12	1.5872	−4.0278	7.2022

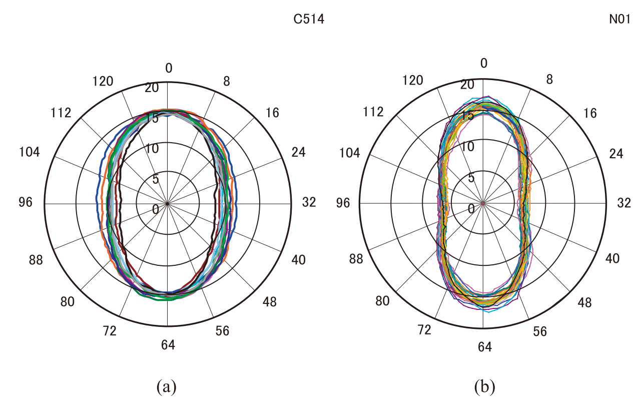

Fig. 10. Example of changes in the basic *Bombyx mori* cocoon shape with cocoon size.
(a) Measured cocoon shapes for silkworm race C514
(b) Measured cocoon shapes for silkworm race N01

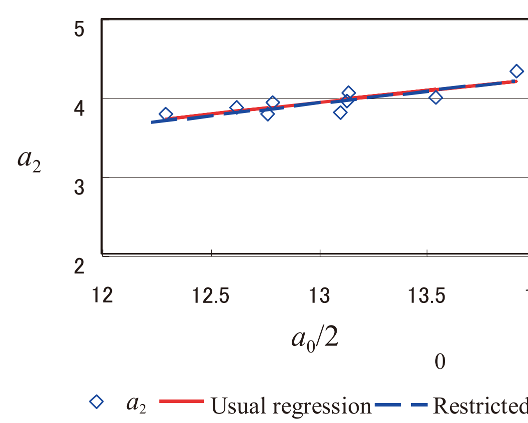

Fig. 11. Regression lines of a_2 against $a_0/2$ for race PCG × N124.

3. Hybrid-type cocoon

Figure 11 shows a plot of the a_2 against $a_0/2$ for the result of a cross between race PCG and N124 (PCG × N124). The regression line for this race indicates that the basic shape of PCG × N124 did not vary with size. Figure 12 shows a similar plot for race N510 × C512. In this case, the basic shape of the cocoon did not vary with size, either. The intercepts of regression for hybridized races are shown in Table 3. The correlation between coefficient $a_0/2$ and a_2 for these races, with the exception of races PCG × N124 and N510 × C512, was poor. In general, most of the

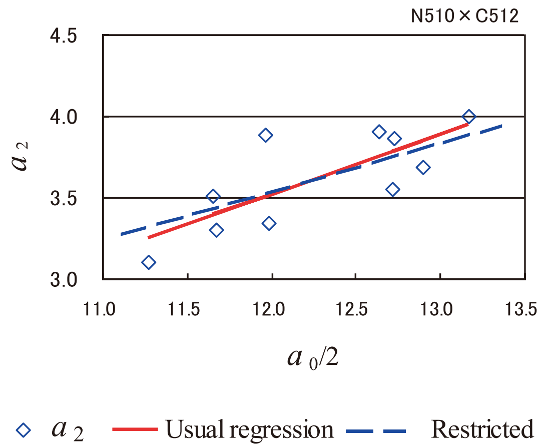

Fig. 12. Regression lines of a_2 against $a_0/2$ for race N510 × C512.

Table 3. Intercepts obtained from general regression and its upper and lower limits in the hybridized-race *Bombyx mori* cocoons

		95% confidence intervals		
races	*n*	intercept	lower limit	upper limit
AKEBONO	12	2.1875	−5.1315	9.5064
N137C146	11	2.5808	−1.3474	6.5089
HONOBONO	10	2.1875	−5.1315	9.5064
PCG × N124	9	0.0748	−2.2711	2.4207
N510 × C512	10	−0.8833	−3.9223	2.1557
PNG × PCSG	10	3.2491	−1.5915	8.0896
PNY × PCSY	10	3.6955	0.6971	6.6938

hybrid cocoon shapes did not noticeably vary with size.

DISCUSSION

The basic shapes of the Japanese silkworm cocoons did not change with cocoon size, as indicated by the length and width coefficients, with the same ratio for various cocoon sizes. However, the basic shapes of some Chinese silkworm cocoons (C108 and C514) change with size, according to the difference in the variation of the length and width of the cocoon.

It should be noted that not all of the Chinese races necessarily have an inherent basic cocoon shape. Enokijima described a similar correlation to those in the present study between the ratio of the cocoon's length to width and the cocoon's volume for each race. However, nothing had been mentioned about it at that time.

We have been studying changes in the basic shapes of cocoons that are associated with a variation in cocoon size within races in order to evaluate the silkworm-breeding environment. We conclude that this phenomenon is evidence that cocoons are generated by living things rather than by machines.

ACKNOWLEDGMENTS

We would like to express our gratitude to Dr. T. Yamamoto, the chief of the former National Institute of Agrobiological Sciences Silk Technology Unit for his advice as to the handling and techniques for silkworm rearing. The silkworm strains used in this study were donated by the National Institute of Agrobiological Sciences, and we wish to express our gratitude. The National Biosource Project (RR2002) and NIAAS Genebank supported the rearing of silkworm larvae.

We would like to thank Editage for providing editorial assistance.

REFERENCES

Enokijima, M., Yamamoto, T., Shimiz, K. and Shimizu, F. (1985) Racial specificity of several quantitative characters in the silkworm stocks (3) Relationship between the cocoon shape and some quantitative characters. Tech. Bull. Sericul. Exp. Sta., 124, 35-45.

Nishioka, T., Satoh, H. and Liew, K. (1998) Numerical representation for cocoon shapes by principal component analysis that uses Fourier coefficients. *J. Sericult. Sci. Jpn*., 67, 479-484 (in Japanese with English summary)

Nishioka, T., Nakazawa, K. and Chow, K. (2001) Decision of number of the order of Fourier series by Akaike Information Criterion of basic shape of the cocoon *J. Sericult. Sci. Jpn*., 70, 11-15 (in Japanese with English summary)

Nishioka, T. Mase, K. and Kajiura, Z. (2010) The dispersion of the cocoon shape and its Mahalanobis's generalized-distance between races. *Journal of Insect Biotechnology and Sericology.,* 79, 9-13.