Development of a Gender Identification Device for Eri Silkworm Pupae

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Abstract. *To know the gender of an Eri silkworm pupa is important because the cocoon from male silkworms can be used to produce the silk with better quality than the cocoon from the female silkworms. The male and female silkworm pupae themselves also contain different medicinal properties. Moreover, to increase efficiency in silkworm breeding, the number of male and female pupae should be equal. Therefore, the aim of the research is to develop a gender identification device for Eri silkworm pupae using digital image processing. The single board computer was adopted as a main controller of the developed device and the Qt program with the OpenCV library was used for programming. First, the pupa was held using the holding mechanism and then a red LED was lit up near the sex mark of the pupa. After that the proposed image processing algorithm was conducted to distinguish between male and female pupae. The experimental results showed that the accuracies of the gender identification for male pupae and female pupae were 92% and 98%, respectively. The overall accuracy was 95%. The average operating time for one pupa was 9.3 s. Then, it was used to identify the gender of pupae for silkworm breeding. The results showed that the number of eggs was increased by 52.2% compared with the cocoon-uncut gender identification method by silkworm farmers. As a result, the gender identification device can be used to identify the gender of the Eri silkworm pupae effectively and increase the efficiency of the silkworm breeding significantly.*

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1. Introduction

Eri silkworms are recognized as an outstanding instance of sustainable agriculture since pupae can be used as a protein food source [1] and cocoons can be used to produce silk. Moreover, they have been used for several biomedical applications [2] and silk pupa protein hydrolysates are one of several ingredients in cosmetics industries [3].

Many researchers have tried to identify the gender of silkworm pupae since many benefits can be gained. For example, Liu et al. [4] and Zhang et al. [5] found that the male silkworm cocoon gives better silk quality than female pupae when the silkworms separated. Oh et al. [6] found that the male silkworm pupa powder (SWP) helps increase sexual performance. Wattanathorn et al. [7] showed that silkworm pupae could attenuate memory impairment and neurode-generation in Alzheimer's disease. Besides, if the number of male cocoons is matched the number of female cocoons, the resulting eggs will increase.

At present, the gender of silkworm pupae is identified by an expert. This process is time-consuming and eyes may get tired easily. The accuracy cannot be consistency, depending on the expert's performance. Therefore, the main contribution to this work is to develop a device that can identify the gender of Eri silkworm pupae quickly and more accurately.

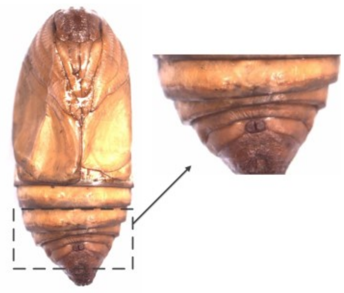
2. Materials and Methods

To design a gender identification device for Eri silkworm pupae, the study of silkworm pupa characteristics was necessary as shown in the following subsection.

2.1 Gender Identification for Eri silkworm pupae

The female silkworm pupa is larger than the male silkworm pupa and the pupa skin color is lighter, and the posterior abdominal segment is more rounded in shape [8]. Ventrally, in the center of the eighth abdominal segment, there is an X-shaped mark extending from the anterior to the posterior margins of the segment, as shown in Fig. 1(a). The male silkworm pupa is smaller than the female and the last abdominal segment is more or less pointed, and in the center of the ninth ventral segment, there is a small brown spot, the Herold's gland, as shown in Fig. 1(b). Since female cocoons are usually larger and heavier than males of the same race, especially among the multivoltine races, use can be made of these differences to separate the female cocoons from the male cocoons by weighing. The weight of cocoons has been used to identify the gender as well [9]. It was called a sexing machine. In general, the female cocoon is heavier than the male cocoon but this assumption is not always correct. The color of eggs and the special mark appeared at the early cocoon stage can also be noticed by an expert [10] but this method is difficult.

(a)



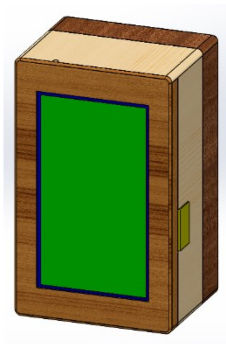
(b)

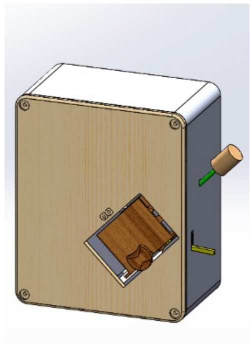
Fig. 1: External shapes and characteristics of the reproductive glands of (a) female (b) male

Sumriddetchkajorn et al. [11] studied the effect of different wavelength spectral in identifying the gender of silkworm pupae. Kamtongdee et al. [12] studied silkworm gender identification with normalized cross correlation (NCC)-based pattern matching operation. Liu et al. [4] developed the water proportion difference inside the silkworm cocoon with imaging of magnetic resonance imaging (MRI). Shenyuan et al. [13] studied difference between male and female of Bombyx mori pupae in near infrared reflectance spectra (NIRS). Cai et al. [14] invented non-destructive gender discrimination of silkworm cocoons with X-ray images .Then, their use four different types, including the K-nearest neighbors, linear discriminant analysis, back-propagation artificial neural network and support vector machine algorithms. In our work, we found that the sex mark of the male pupa is opaquer to the red LED than that of the female pupa. Thus, we developed a device to hold a pupa and analyzed the sex mark (see Fig. 1) using an image processing algorithm.

2.2 The Gender Identification Device

The device is designed to be compact, portable and easily-used. It consists of two units (see Fig. 2): a display unit and a pupa image capture unit.





(a) (b)

Fig. 2: Design of a gender identification device (a) a display unit (b) a pupa image capture unit

The display unit consists of a touchscreen used to show the gender identification result and parameter settings. There are 2 layers inside the display unit (see Fig. 3). The Raspberry Pi board, a voltage regulator and a voltage indicator are located at the upper layer, while two 11.1VDC 3700 mAh batteries and an on/off switch are mounted at the lower layer.

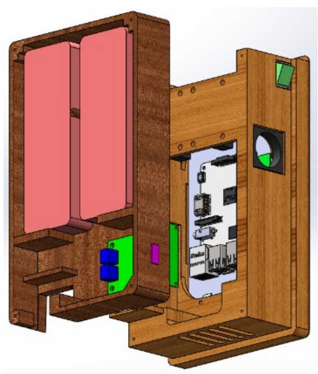


Fig. 3: Component arrangement inside the display unit

There is an opening cover on one side of the pupa image capture unit. This cover is used to prevent the illumination from outside. The pupa is taken into the pupa holder mechanism inside the pupa image capture unit and it is then locked (see Fig. 4). The pupa can be rotated using a rotating knot in order that the pupa image is taken in the correct orientation. A 5MP webcam is mounted and pointed to the bottom of the pupa. The distance from the webcam to the pupa is about 5 cm. A red LED is attached at the angle of 45 degrees with respect to the pupa body. The light goes through the pupa body.

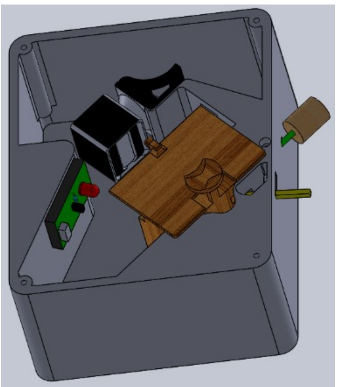


Fig. 4: Component arrangement inside the pupa image capture unit

After the image of the bottom-up pupa is captured (see Fig. 5(a)), the following image processing algorithm is performed:

(1) The image shown in Fig. 5(a) is cropped around the bottom of the pupa shown in Fig. 5(b)

(2) The cropped image is converted to the gray-scale image and then to the inverted binary image using the user-defined inverse binary thresholding value (see Fig. 5(c) in case of a female pupa)

(3) A line is drawn from the bottom-center position of the image (black region) to the edge of which intensity is changed (black to white edge). Each line is apart 6 degrees from each other (see Fig. 5(d) in case of a female pupa).

(4) The length of the longest line is subtracted by the length of the shortest line and is divided by the length of the longest line (see Eq. (1)).

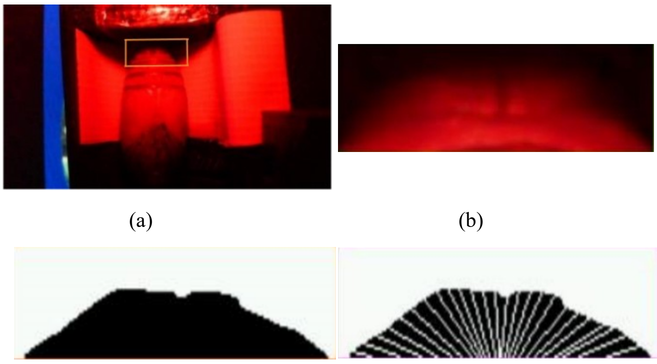
(5) If the resulting value is greater than 0.55, the pupa is identified as male, otherwise, it is identified as female.

$$\frac{\text{Max\_Length} - \text{Min\_Length}}{\text{Max\_Length}} > 0.55 \tag{1}$$

The number (0.55) given in Eq. (1) is from the experiment. We used a hundred of pupae and followed the above image processing algorithm. The length of the longest line and length of the shortest line were measured and then we obtained the optimal number based on the minimum distance classifier. The user-defined thresholding value is also important since this value has an impact on the accuracy of the device. The improper thresholding value results in wrong segmentation and then wrong identification. In general, the range of this value is from 20 to 50 (the maximum value is 255). It is obtained by experiment.

Fig. 6 shows the user interface developed under the Qt environment:

- No. 1: Start/Run button for image capture
- No. 2: Detect button to identify the gender
- No. 3: Gender identification
- No. 4: RGB image of pupa
- No. 5: Gray-scale image
- No. 6: Binary image (segmentation)
- No. 7: Analyzed image with lines
- No. 8: Slider Bar for specifying the thresholding value
- No.9 : Exit button to exit the program



(a) (b)

(c) (d)

Fig. 5: Image processing algorithm: (a) pupa image was captured, (b) a cropped image, (c) an inverse binary image (segmentation), and (d) lines on the binary image to identify the gender of the pupa

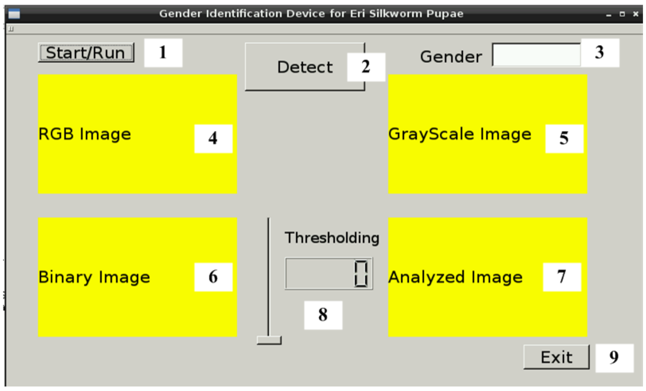


Fig. 6: The user interface developed under the Qt environment

3. Experimental Results

The developed gender identification device is shown in Fig. 7. To evaluate the performance of the developed device, one hundred male and one hundred female pupae were supplied by Silk Innovation Center, Mahasarakham University. First, 20 samples were inserted into the pupa image capture unit (see Fig. 8), one by one. The image of the pupa was captured as shown in Fig. 9. Then, the user adjusted the optimal threshold value that was able to extract the pupa from the background. In this experiment, the final threshold value was 35 (the maximum value was 255). The experimental results are shown in Table 1. The example of the resulting gender identification is shown in Fig. 10.

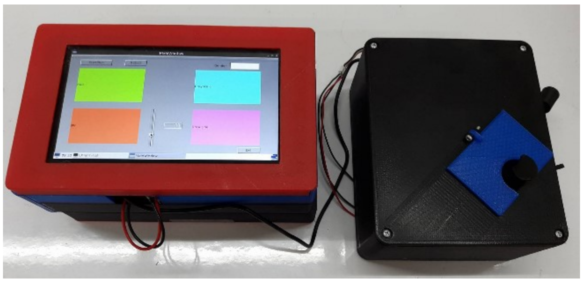


Fig. 7: The gender identification device

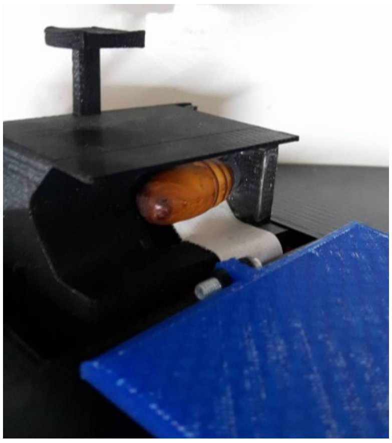


Fig. 8: The pupa inserted into the pupa image capture unit

Fig. 9: The image of the pupa inside the image capture unit

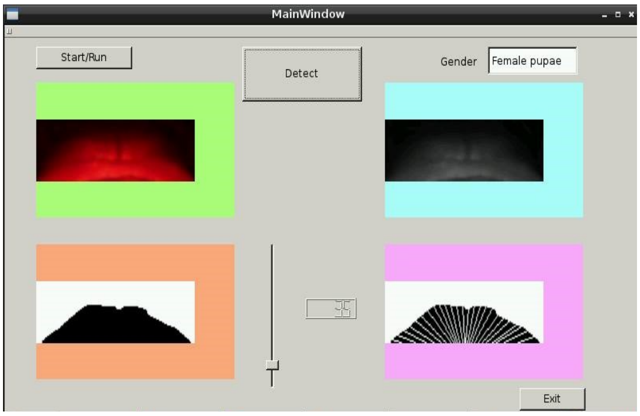


Fig. 10 : The resulting gender identification

Pupae	The proposed device		% Accuracy
	Male	Female	
Male	92	8	92
Female	2	98	98
Average			95

Table 1 The accuracy of gender identification using the developed device

User	No. of Pupa	Total Operating Time (minutes)	Operating time per one pupa (seconds)
Researcher	200	27	8.1
Unexperienced User 1	200	35	10.5
Unexperienced User 2	200	31	9.3
Average		31	9.3

Table 2 The operating time

As seen in Table 1, the average accuracy was 95%. The main error was caused by the unclear sex mark of the pupa. This resulted in wrong segmentation. The next experiment was to measure the operating time. Two hundred pupae were used in this experiment. The timer was started when the first pupa was inserted into the device and the timer was stopped when the last pupa was taken out of the device. There were three users and the result is shown in Table 2. We found that the operating time for one pupa was 9.3 seconds or 387 pupae per for hour.

In the last experiment, the number of eggs was compared when the silkworm pupae were matched for breeding. Traditionally, the male and female pupae were matched, based on the experience. Fig. 11 shows the breeding house used to collect eggs. The male/female matching was performed by the researcher with the developed device and the farmer who used a traditional method. Each took 10 male/female pair.

Fig. 11: The breeding house

The number of eggs was counted as shown in Fig. 12. We found that the number of eggs, based on two methods above, were 988 and 649 eggs, respectively. Therefore, the number of eggs was increased by 52.2% compared with the cocoon-uncut gender identification method by silkworm farmers.

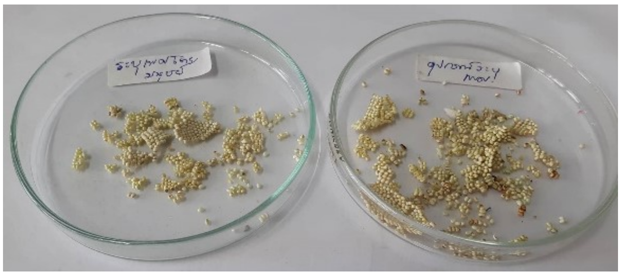


Fig. 12: The eggs from male/female matching experiments

Finally, the cost of the device is 14,000 Baht. As given by the Queen Sirikit Department of Sericulture, one sheet of eggs (about 20,000 eggs) costs 300 Baht. Therefore each egg cost about 0.015 Baht. We can increase 52.2% egg producing. Thus, we will gain 156 Baht per one sheet. In general, five sheets of eggs can be produced each month (Silk Innovation Center, Mahasarakham University), resulting in 780 Baht.

4. Conclusions

The gender identification device developed in this work consisted of 2 units: (1 the display unit and (2 the pupa image capture unit. This device is compact and portable. The unexperienced user can use this device to identify the gender of the Eri silkworm pupa easily. The experimental results showed that the average accuracy was 95% and the operating time for each pupa was 9.3 seconds.

Then, the developed device was used to identify the gender of pupae when a male/female pair was prepared for breeding. The experimental results showed that the number of eggs was increased 52.2% compared to the traditional method. Thus the novelty of this work is the developed device that can reduce the labor cost and increase the accuracy. In the future work, we would like to develop an automatic thresholding algorithm in order to increase the accuracy and reduce the number of setting parameters.

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References

[1] Wang, W., Shen, S., Chen, Q., Tanga, B., He, G., Ruan, H., Das, U.N. Hydrolyzates of silkworm pupae (Bombyx Mori) protein is a new source of angiotensin I-converting enzyme inhibitory peptides (ACEIP). *Current Pharmaceutical Biotechnology*, 2008, vol. 9, no. 4, p. 307-314.

[2] Singh, K.P., Jayasomu, R.S. Bombyx mori – A review of its potential as a medicinal insect. *Pharmaceutical Biology*, 2002, vol. 40, no. 1, p. 28-32.

[3] Kato, N., Sato, S., Yamanaka, A., Yamada, H., Fuwa, N., Nomura, M. Silk protein, Sericin, inhibits lipid peroxidation and tyrosinase activity. *Bioscience, Biotechnology, and Biochemistry*, 1998, vol. 62, no. 1, p. 145-147.

[4] Liu, C., Ren, Z.H., Wang, H.Z., Yang, P.Q., Zhang, X.L. Analysis on Gender of Silkworms by MRI Technology. *In Proceedings of the 1st International Conference on BioMedical Engineering and Informatics*, 2008, p. 8 - 12.

[5] Zhang, Y.Q., Yu, X.H., Shen, W.D., Ma, Y.L., Zhou, L.X., Xu, N.X. Yi, S.Q. Mechanism of fluorescent cocoon sex identification for silkworms Bombyx mori. *Science China*, 2010, Vol. 53, no. 11, p. 1330-1339.

[6] Oh, H.G., Lee, H.Y., Kim, J.H., Kang, Y.R., Moon, D.I., Seo, M.Y., Park, J.K. Effects of male silkworm pupa powder on the erectile dysfunction by chronic ethanol consumption in rats. *Laboratory Animal Research*, 2012, Vol. 28, no. 2, p. 83-90.

[7] Wattanathorn, J., Muchimapura, S., Boosel, A., Kongpa, S. Kaewrueng, W., Tong-Un, T., Thukhammee, W. Silkworm pupae protect against Alzheimer's disease. *American Journal of Agricultural and Biological Sciences*, 2012, Vol. 7, p. 330-336.

[8] Chinnakotr, J., Khongcharoen, N., Kanjanawanishkul, K., Lasunon, O.U. Experiments on Imaging Parameters for Identification of Male Silkworm Pupae. *In Proceedings of the 13th Mahasarakham University Research Conference*, 2017, p. 326 - 334.

[9] Wang, S.M. *Silkworm egg production*. 3rd ed. Food and Agriculture Organization of the United Nations, 1989.

[10] Nagaruju, J. Sex determination and sex-limited traits in the silkworm, Bombyx mori: Their applications in sericulture. *Indian Journal of Sericulture*, 1996, Vol. 35, no. 2, p. 83-89.

[11] Sumriddetchkajorn, S., Kamtongdee, C., Sa-Ngiamsak, C. Spectral imaging analysis for silkworm gender classification. *In Proceedings of the 14th SPIE Conference on Sensing Technologies for Biomaterial, Food, and Agriculture*, 2013.

[12] Kamtongdee, C., Sumriddetchkajorn, S., Sa-Ngiamsak, C. Feasibility Study of silkworm pupa sex identification with pattern matching. *Computers and Electronics in Agriculture*, 2013, Vol. 95, p. 31-37.

[13] Shenyuan, P., Ming, T., Aiqun, S., Tongming, J. Difference between male and female pupae of Bombyx mori in near infrared reflectance spectra and their pattern recognition. *Acta Entomologica Sinica*, 1996, Vol. 39, no. 4, p. 360-365.

[14] Cai, J.R., Yuan, L.M., Liua, B., Sun, L. Nondestructive gender identification of silkworm cocoons using X-ray imaging with multivariate data analysis. *Analytical Methods*, 2014, Vol. 6, no. 18, p. 7224-7233.

Biographies

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