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Elevation Affects the Development, Growth, and Cocoon Quality of Eri Silkworm *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae)

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ABSTRACT

Samia cynthia ricini is a species of silkworm whose survival, development, and cocoon quality are affected by its environmental factors such as elevation, which affects temperature and humidity. This study aimed to examine the impact of elevation on the survival, development time, cocoon shell weight, and fecundity of *S. c. ricini*. The research was performed at two sites with different elevations: highland and lowland regions. The findings indicated that *S. c. ricini*, reared in the highlands, had higher survival rates than those cultivated in the lowlands. Meanwhile, the developmental time of *S. c. ricini* reared in the lowlands was faster than in the highlands. Additionally, the cocoon shell weight of *S. c. ricini* reared in the lowlands was also heavier, and the total fecundity of the adults of *S. c. ricini* reared in the lowlands was higher than those reared in the highlands. This study recommends that *S. c. ricini* be reared in lowland settings for best results.

1. Introduction

Samia cynthia ricini is a commercial silk-producing insect originating from India (Jolly 1981) and is currently spread across several countries such as China, Japan (Peigler 1993), Korea (Kim *et al*. 2012), Kenya (Oduor *et al*. 2021), Ethiopia (Pallabi and Sharma 2017), Thailand (Tungjitwitayakul and Tatun 2017), and Indonesia (Trisnawati and Nurkomar 2020). *Samia cynthia ricini* is an oligophagous insect (Nangia *et al*. 2000) that primarily feeds on castor leaves (Sirimungkararat *et al*. 2008; Nurkomar *et al*. 2022a), while its secondary food source is cassava leaves (Nurkomar *et al*. 2022b).

Diet sources and environmental conditions are essential for the silkworm life cycle (Atmosoedarjo *et al*. 2000). In controlled laboratory settings, *S. c. ricini* exhibited a high survival rate when fed on castor leaves. A notable difference in the total larval development time was observed, with larvae developed for 18 days on castor and 21 days on cassava, while the pupa and cocoon shell weight were higher when raised on castor leaves, and adult females laid more eggs (300-500) when feeding on castor during the larval stage. (Nurkomar *et al*. 2022a; Das and Das 2018).

Silkworms are cold-blooded organisms (poikilothermic) whose development is greatly influenced by environmental conditions (Ouisse *et al*. 2020; Zhang *et al*. 2021). As an initial step in the national assessment of *S. c. ricini* production regions, it is necessary to conduct research on the survival, development time, cocoon shell weight, and fecundity of *S. c. ricini* under different regional conditions, such as varying elevations. However, how environmental conditions affect the biological parameters of *S. c.*

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ricini is still unknown. So far, research has been limited to the effects of diet types (Nurkomar *et al*. 2022a; Nurkomar *et al*. 2022b), modifications of the life cycle (Nurkomar *et al*. 2023), and population density (Nurkomar *et al*. 2024) on survival, development time, cocoon weight, and fecundity of *S. c. ricini*. This study aimed to investigate the effect of elevation on survival, development time, cocoon weight, and fecundity of *S. c. ricini*.

2. Materials and Methods

2.1. Leaves Diet

The leaves used as a diet source are castor leaves (*Ricinus communis*). The leaf diet is obtained from the cultivation area owned by a company (CV. KUPU Sutra) in Pasuruan, East Java, Indonesia. The leaves are cleaned with tap water and dried using tissue paper before being used as a diet. The leaves are provided from instar 1 to instar 5. The first and second instar larvae are fed 2-3 leaves, while the third to fifth instar larvae are fed 8-10 leaves per larval rearing container. The diet is provided using the Ad Libitum method.

2.2. Development, Growth, and Cocoon Quality of *Samia cynthia ricini* at Different Elevations

The study was conducted by rearing *S. c. ricini* on farms owned by company-assisted breeders in regions with different elevations as treatments, i.e., in Batu City for the highland region (900 m above sea level) with daily temperature ranging from 17-30°C and relative humidity of 34-87% and Bangil District, Pasuruan Regency, for the lowland region (45 m above sea level) with daily temperature ranging from 26-38°C and relative humidity of 38-79%. Each treatment involved 30 *S. c. ricini* individuals. This number was determined based on the minimum population density for rearing *S. c. ricini* (Nurkomar *et al*. 2024). Each treatment was repeated three times. In total, 90 individuals of *S. c. ricini* were used in each treatment.

2.3. Rearing Process

The rearing starts from the egg and continues until the adult stage. The eggs were obtained from CV. KUPU Sutra, Pasuruan, East Java, Indonesia. Research began by preparing 90 eggs for each treatment. The eggs were kept in Petri dishes (diameter = 83 mm). The eggs were observed daily to count the number of hatchlings. Newly hatched larvae (instar 1) were transferred into larval rearing containers (32 × 25 × 5 cm) lined with paper. The leaf diet was prepared inside these containers. The larvae were reared until instar 5. As they approach the prepupal stage, the larvae typically search for a place to pupate, so the late-stage instar five larvae were moved into a place for pupation. After the cocoons had formed, the pupae were harvested and weighed. The pupae were then transferred to adult rearing cages (37 × 30 × 33 cm) and maintained until the adults died. The parameters measured were survival rate, development time (egg, larva, pupa, adult), weight of fifth instar larvae, pupal weight, cocoon shell weight, and adult fecundity.

2.4. Data Analysis

The survival of *S. c. ricini* was analyzed using Kaplan-Meier's Survival Analysis (Dudley *et al*. 2016). Other parameter data were analyzed using paired *t*-tests. Data analysis was performed using R Statistical Software ver. 4.2.2 (R Core Team 2015).

3. Results

The study results indicate that elevation affects the survival of *S. c. ricini* ($p<0.0001$). Initially, *S. c. ricini* reared in highland and lowland regions had the same survival rate from egg to instar 4, with the entire population remaining alive (Figure 1). The survival of *S. c. ricini* reared in the highlands decreased to 90% at instar 5, while those in the lowlands decreased to 97.5% and 90% at the pupal stage. No survival decrease was observed from instar 5 to the pupal stage in *S. c. ricini* reared in the highlands. However, only 85% of the pupae from the highland rearing successfully developed into adults, compared to only 67.5% of the pupae from the lowland rearing successfully developed into adults (Figure 1).

In addition to survival, the development time of *S. c. ricini* reared in the highlands also differed significantly from those reared in the lowlands. The egg stage showed no significant difference in development time between the two elevations. However, during the larval stage, *S. c. ricini* reared in the lowlands developed four days faster than those in the highlands ($p<0.0001$, paired *t*-test). Similarly, in the pupal stage, individuals reared in the lowlands emerged one day earlier than those in the highlands (Table 1). The adult stage also exhibited a significant difference, with *S. c. ricini* from the lowlands reaching maturity sooner than those in the highlands. Overall, the total development time from egg to adult was five days faster in the lowlands than in the highlands ($p<0.01$, paired *t*-test).

Samia cynthia ricini larvae reared in the lowlands were more active in consuming the provided diet than those reared in the highlands. The increased feeding activity of *S. c. ricini* in lowland environments is evidenced by the final instar larvae's weight, significantly greater than that of larvae reared in highland conditions (p<0.01, paired t-test). The pupal and cocoon shell weights of *S. c. ricini* reared in the lowlands were significantly greater than those of individuals reared in the highlands (Table 2). Moreover, the difference in pupal weight of *S. c. ricini* also affects the fecundity of the adults. *S. c. ricini* adults reared in the lowlands were able to produce more eggs than those reared in the highlands significantly (p<0.01, paired t-test) (Table 2).

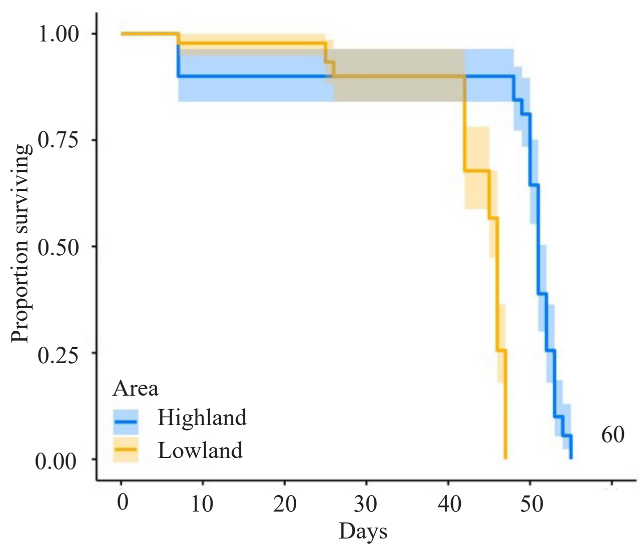

Figure 1. Survival of *Samia cynthia ricini* reared in highland and lowland regions. Shading area indicates the standard error value

Table 1. Life cycle of *Samia cynthia ricini* in the highland and lowland regions

Stadia	Development time (mean ±SD) (days)		p value
	Lowland	Highland	
Egg	7.000±0.000	7.000±0.000	-
Larvae			
1st instar	3.000±0.000	3.000±0.000	-
2nd instar	3.000±0.000	3.000±0.000	-
3rd instar	3.000±0.000	4.000±0.000	-
4th instar	3.000±0.000	3.000±0.000	-
5th instar	6.261±0.441	6.814±0.390	0. 001*
Total Larva	18.261±0.441	22.814±0.390	2.35 e-05*
Pupae	16.395±0.584	17.667±0.915	0.003*
Adult	4.688±0.466	4.397±0.571	0.037*
Total	42.788±7.811	47.000±13.508	0.0023*

Data shows mean±standard deviation. Asterisk indicates a significant difference based on paired t-tests (α = 5%).

Table 2. Cocoon shell, pupal weight, adult female fecundity of *Samia cynthia ricini* reared in highland and lowland regions

Parameters	Lowland	Highland	p value
Pupal weight	3.211±0.320	1.936±0.637	0.002*
Cocoon shell weight	0.430±0.033	0.230±0.154	0.003*
Number of eggs	289.130±6.328	217.686±24.299	0.015*

Data shows mean±standard deviation. Asterisk indicates a significant difference based on paired t-tests (α = 5%).

4. Discussion

Elevation is a key environmental factor as it generally results in lower temperatures and changes in humidity (Körner 2007), both of which can significantly impact insect development, including silkworm (Hussain *et al*. 2011). Optimal temperature is crucial for proper larval growth, and extreme fluctuations may induce stress, prolong development time, and reduce cocoon quality. Additionally, humidity is vital in maintaining silk gland function, influencing silk production, and preventing desiccation or fungal infections during cocooning. Given these potential impacts, we hypothesize that elevation significantly affects the survival, development time, cocoon weight, and fecundity of *S. c. ricini*, with individuals reared at different elevations exhibiting variations in these biological parameters.

Our study showed that the survival of *S. c. ricini* in the highlands is higher than in the lowlands. The difference in survival rates may be due to variations in environmental conditions, such as temperature and humidity, which directly affect their physiology. Environmental factors, particularly temperature and humidity, have been found to significantly impact the survival rate of *Bombyx mori* in Bangil District, with thermal treatment during each larval instar reducing survival rates (Samsijah and Kusumaputra 1975; Kato *et al*. 1998). Physiologically, temperatures exceeding 35°C can lead to cellular stress and impaired metabolic functions in S. ricini, a relative of *S. c. ricini* (Sahu *et al*. 2006). The decrease in survival of *S. c. ricini* from pupa to adult when reared in the lowlands occurs because the pupae and adults of *S. c. ricini* cannot tolerate excessively high temperatures. Prolonged exposure to high temperatures, such as those in the Bangil District, which can reach 38°C during the day, may cause heat-induced protein denaturation and oxidative stress in pupae, leading to mortality before adulthood or preventing adults from emerging from the cocoon. High temperatures can also impair endocrine regulation, which is critical for the molting and emergence processes.

The development time *S. c. ricini* reared in the lowlands was significantly faster than in the highlands. This difference is closely related to temperature, as the highland temperatures are more moderate than the higher temperatures in the lowlands. Insects generally develop faster at higher temperatures due to accelerated metabolic and enzymatic activities (Subedi *et al*. 2023). Each species has a defined thermal tolerance range for development. Beyond this range, physiological disruptions occur, and development ceases. For instance, the larvae of *Chrysomela aeneicollis* reared in the highlands developed more slowly than those in the lowlands due to cooler temperatures (Dahlhoff *et al*. 2019). Similarly, the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), developed at varying rates within a temperature range of 19-33°C but could not complete its life cycle at 16°C and 36°C (Ju *et al*. 2011). These variations are attributed to temperature-sensitive physiological processes such as enzyme activity, energy metabolism, and hormonal regulation.

Inadequate temperature and humidity that do not meet the needs of the silkworm for development can cause a lack of appetite, leading to underfeeding, which makes the silkworms more susceptible to diseases and hinders their growth (Purwanti 2007). The larvae of *S. c. ricini* reared in the lowlands experience faster molting than those in the highlands because higher temperature is more suitable for metabolic rates and developmental processes. Although the exact molting duration was not measured, it correlates with the accelerated development time of larvae in lowland regions relative to highland regions. Besides impacting development time, variations in environmental conditions between highlands and lowlands also affect the behavior of *S. c. ricini*. Comparable effects have been noted in various other insect species. For example, the Sitona gressorius beetle increases its activity at elevated temperatures compared to intermediate temperatures (Hannigan *et al*. 2023). Beyond its effect on individual development, altitude influences various aspects of insect life, including community structure and diversity (Zhao *et al*. 2023), flight performance (Dillon *et al*. 2006), and seasonal migration (Huang *et al*. 2024). These broader ecological impacts highlight the significance of elevation as a key environmental factor shaping insect biology.

Samia cynthia ricini reared in the lowlands, developing heavier pupae and exhibiting more voracious feeding behavior than those in the highlands. This increased body mass enhanced their ability to produce silk, as silk production is directly related to the amount and frequency of feeding. Consequently, the greater silk yield resulted in heavier cocoon shells as it has been studied in *Bombyx mori* (Atmosoedarjo *et al*. 2000). Similarly, cocoon weight variations in silkworms are affected by their capacity to accumulate silk-forming proteins during instars 4 and 5 (Cholifah *et al*. 2012), as well as factors like silk production capacity, species, diet quality, and environmental conditions such as temperature and humidity during the pupal stage (Hartati and Umar 2012).

Finally, elevation affects *S. c. ricini* fecundity since *S. c. ricini* residing in lowland regions can generate a more significant number of eggs than those in highland areas. The higher feeding activity of *S. c. ricini* in the lowlands leads to a higher protein content, which leads to improved development, fecundity of organisms, and several eggs produced (Lestari *et al*. 2013). Several studies have also reported a positive relationship between cocoon weight and pupal weight, as well as between pupal weight and egg production (Gowda *et al*. 1989; Jayaswal *et al*. 1993).

This study implies cultivating *S. c. ricini* in lowland environments for optimal results, as they develop faster, produce heavier cocoon shells, and exhibit higher fecundity compared to those reared in highlands. The difference in larval development duration also has practical implications for rearing, including diet provision and resource efficiency. Slower development at cooler temperatures reduces dietary requirements and rearing costs, while faster development at optimal temperatures allows for earlier cocoon harvesting. However, extreme temperatures outside the thermal tolerance range can disrupt critical physiological processes, ultimately impacting survival, growth, and silk production.

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