Developmental Biology, Survival and Morphometric Progression of Papilio demoleus Under Laboratory Conditions

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DOI: https://doi.org/10.51244/IJRSI.2026.1310000085

Received: 14 October 2026; Accepted: 20 October 2026; Published: 02 November 2026

ABSTRACT

Developmental information is useful for interpreting the life history of citrus-feeding swallowtails and for identifying the stages at which mortality and growth are concentrated. The developmental biology of Papilio demoleus was studied under laboratory conditions using a field-derived culture maintained at 27 ± 2 °C, 70 ± 5% relative humidity and a 14 h light:10 h dark photoperiod. Fresh Citrus limon foliage was supplied to larvae and a 10% honey solution was provided to adults. Of 360 eggs followed individually, 276 hatched, giving 76.67% hatchability, and 163 individuals completed development to successful adult emergence, equivalent to 45.28% overall egg-to-adult survival. Mean egg incubation was 2.84 ± 0.036 days (SE). Five larval instars were recorded. Mean instar durations were 2.78, 2.76, 2.83, 3.50 and 4.59 days, respectively, and total larval development averaged 16.57 ± 0.110 days (SE; n = 191). Survival remained above 90% through each larval transition, with the highest stage-specific survival during the prepupal transition (97.91%). Larval size increased progressively: mean body length increased from 3.61 ± 0.019 mm in the first instar to 43.40 ± 0.139 mm in the fifth instar, while body width increased from 1.55 ± 0.008 to 6.90 ± 0.022 mm. The prepupal period averaged 1.02 ± 0.009 days and successful pupae required 7.98 ± 0.084 days before adult emergence. Total immature development from egg to successful adult emergence averaged 28.34 ± 0.169 days (SE; n = 163). Pupal emergence was 87.17%. Mean wingspan was 89.90 mm in males and 90.90 mm in females. Female longevity (6.99 ± 0.093 days) exceeded male longevity (4.29 ± 0.103 days). Reproductively active females produced 106.40 ± 1.993 eggs on average. These findings provide an integrated laboratory reference for developmental duration, stage-specific survival and morphometric progression in a central Indian population of P. demoleus, while demonstrating how cumulative losses across successive stages reduce overall egg-to-adult survival.

Keywords: Papilio demoleus; developmental biology; larval instars; morphometrics; survival; fecundity; life cycle; Lepidoptera

INTRODUCTION

Developmental biology provides the basic framework for understanding how an insect moves from one life stage to the next, how rapidly it grows and when mortality is most likely to occur. In holometabolous insects, the egg, larval, pupal and adult stages differ greatly in structure, behaviour and ecological function. The larva is primarily a feeding and growth stage, the pupa reorganizes larval tissues into the adult form, and the adult is mainly responsible for dispersal and reproduction. These stage differences also mean that temperature, food quality and other rearing conditions can affect each part of the life cycle differently (Kingsolver & Buckley, 2020; Buckley, 2022). Standardized laboratory observations are therefore useful because they allow stage duration, survival and body measurements to be recorded under a common set of conditions.

The lime swallowtail, Papilio demoleus Linnaeus (Lepidoptera: Papilionidae), is one of the most widely distributed swallowtails associated with citrus-growing regions. Its northern subspecies commonly uses plants in the family Rutaceae, and larvae are well known from Citrus species and related hosts (Riaz et al., 2020). The species is also notable for its ecological adaptability, geographic expansion and importance as a citrus-associated pest, with its biology and management reviewed by Sarada et al. (2014). Populations have

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

established outside parts of their historical range, including the New World, where the citrus-feeding form has been regarded as a potential agricultural pest (Eastwood et al., 2006). This combination of broad distribution, host use and rapid development has made P. demoleus a frequent subject of biological and applied entomological studies.

In India, the species has long been recognized as a citrus caterpillar. Early work described its development and damage on citrus, and later laboratory studies quantified stage duration, adult longevity and morphometric changes under different host and environmental conditions (Atwal, 1964; Ramakrishna Rao, 2015; Patel et al., 2017; Jahnavi et al., 2018). The biological values reported across studies are not identical. Egg, larval and pupal durations can differ among locations and rearing conditions, and experimental temperature is particularly important because insect developmental rate is temperature sensitive (Badawi, 1981; Islam et al., 2019; Kingsolver & Buckley, 2020). These differences make locally generated biological data useful rather than redundant.

Egg hatchability is an important early measure because it determines how many individuals enter the feeding stage. Egg development also provides a clear transition from oviposition to establishment of the first instar. Previous work on P. demoleus has reported incubation periods ranging from a few days to longer periods depending on temperature and study location (Munir et al., 2007; Karim et al., 2007; Tien, 2018; Islam et al., 2019). A laboratory culture in which individual eggs are tracked allows hatchability, incubation time and early losses to be recorded without relying only on field presence or adult abundance.

The larval phase is especially important for both biology and applied entomology because it combines repeated moulting with a large increase in body size and food consumption. P. demoleus normally passes through five larval instars in standard development, although occasional additional instars have been reported under particular conditions. Morphology also changes markedly. Early instars are dark and resemble bird droppings, whereas the mature fifth instar is predominantly green and much larger. Direct studies on lemon, acid lime and other citrus hosts have documented progressive increases in larval dimensions and longer feeding periods in later instars (Patel et al., 2017; Jahnavi et al., 2018; Dharavath et al., 2021). Measuring body length, body width and head-capsule width therefore gives a quantitative description of growth in addition to the visible colour changes used to identify instars.

Survival across developmental stages is equally important. A high survival percentage within a single instar does not necessarily produce high egg-to-adult survival because losses accumulate sequentially. Eggs may fail to hatch, larvae may die during feeding or moulting, prepupae may fail to complete attachment and pupae may die before or during eclosion. Laboratory studies can separate these transitions and identify where the greatest proportional losses occur. Such information is useful for maintaining cultures and for understanding which stages may be most vulnerable under field conditions, although laboratory survival cannot be assumed to represent natural mortality exactly (Badawi, 1981; Riaz et al., 2020).

Pupal and adult traits complete the developmental picture. Pupal duration and dimensions describe the transition between larval growth and adult emergence and provide quantitative characteristics of this non-feeding stage. Adult wingspan, longevity and reproductive output describe the final life-history stage. Previous studies have reported sex differences in adult longevity and substantial variation in fecundity among P. demoleus populations and rearing systems (Karim et al., 2007; Patel et al., 2017; Tien, 2018). These differences may be influenced by adult nutrition, temperature, host-plant effects during larval development and the conditions under which mating and oviposition are observed.

Recent work continues to show that developmental morphology and timing in P. demoleus vary across environments and regions, supporting the value of locally standardized measurements (Bais et al., 2026). Central India has a strongly seasonal climate, but detailed laboratory datasets that combine hatchability, stage-wise survival, larval morphometrics, pupal measurements, adult size, longevity and reproductive performance within the same tracked culture remain useful for comparison with studies from other regions. The present study therefore examined the developmental biology of P. demoleus under laboratory conditions, with emphasis on stage-wise developmental duration, survival, morphometric progression, pupal characteristics, adult emergence, longevity and reproductive parameters.

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MATERIALS AND METHODS

Study Location and Laboratory Conditions

The work formed part of a three-year PhD investigation conducted from July 2023 to June 2026 at Government Holkar Science College, Bhawarkuan, Indore, Madhya Pradesh, India. Field material used to initiate the culture was collected from the Botanical Garden and Butterfly Park, while rearing and developmental observations were conducted in the Department of Zoology. The laboratory culture was maintained at 27 ± 2 °C, 70 ± 5% relative humidity and a 14 h light:10 h dark photoperiod. Temperature and relative humidity were recorded daily. Adult cages were kept away from direct drafts and intense sunlight.

Source and Establishment of Papilio demoleus Culture

Eggs and larvae were collected from unsprayed host plants within the college campus and transported in labelled ventilated containers. Field material was examined before introduction to the culture. Individuals showing visible disease, severe injury or active parasitoid emergence were separated. Across the culture-establishment phase, 520 eggs and 310 larvae were collected, and 726 individuals were successfully introduced after screening. Twelve cohorts were maintained in 24 rearing containers. A subset of 360 eggs was followed for individual developmental observations from egg through adult emergence. Fresh lemon (Citrus limon) foliage was used as the standardized larval food.

Egg Collection and Incubation

Leaves carrying eggs were transferred with a short section of petiole or stem so that the egg surface was not damaged. When movement of an egg was necessary, a fine moistened brush was used. Eggs were maintained singly or sufficiently separated in clean Petri dishes lined with slightly moistened filter paper. Excess free water was avoided. Eggs were examined daily under a stereomicroscope for colour, surface integrity and developmental change. Incubation period was recorded from oviposition, or collection of a newly laid egg, to larval emergence. Empty chorions and unhatched eggs were examined before disposal.

Larval Rearing

First- and second-instar larvae were reared on tender lemon leaves and handled only with a soft brush where movement was necessary. Third- to fifth-instar larvae were given progressively larger amounts of fresh foliage. Larvae were maintained at approximately 15–20 individuals per ventilated container, and fresh leaves were supplied daily. Old foliage, frass, exuviae and dead individuals were removed each day, and containers were washed and dried before reuse. Instar identity was based on head-capsule size, external colour pattern, body size, feeding behaviour and the presence of shed cuticle or head capsules. Each larva was checked daily and the date of each moult was recorded. Instar duration was calculated from completion of one moult to completion of the next.

Prepupal and Pupal Maintenance

Mature fifth-instar larvae were recognized as prepupae when feeding declined, wandering increased, the gut was evacuated and a firm attachment site was selected. Once attached by the posterior silk pad and supporting girdle, individuals were disturbed as little as possible. Newly formed pupae were labelled by cohort and date. Pupal length and maximum width were measured with a digital caliper accurate to 0.01 mm. Pupae were maintained with adequate vertical space for normal adult emergence and wing expansion. Pupal duration was recorded from completed pupation to successful adult emergence.

Adult Emergence and Maintenance

Cages were checked daily for adult emergence. Newly emerged butterflies were not handled until the wings had expanded and hardened. Sex was determined from external adult characters and the emergence date was recorded. Adults were maintained in 60 × 60 × 60 cm nylon-mesh cages and provided with freshly prepared

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10% honey solution on absorbent cotton, replaced every 24 h. Longevity was recorded from emergence to death. When mating and oviposition occurred, the date of mating, first and last oviposition and the number of eggs laid were recorded. Mating duration and oviposition behaviour were recorded as direct observations.

Biological Parameters

The biological variables used in this article were egg incubation period, egg hatchability, duration of each larval instar, total larval duration, prepupal duration, pupal duration, stage-specific survival, overall egg-to-adult survival, adult emergence, adult longevity and reproductive output. Hatchability was calculated from the number of eggs producing living first-instar larvae relative to the number followed. Stage-specific survival was calculated from the number entering a stage and the number successfully reaching the next stage. Fecundity was the total number of eggs laid by an observed female during her recorded reproductive life.

Total larval duration was calculated separately for each individual that successfully completed all five larval instars (n = 191). The recorded durations of the first, second, third, fourth and fifth instars were summed for each complete individual record, and the mean, standard deviation and standard error were calculated from these individual totals. Instar-specific durations were calculated using all individuals that successfully completed the respective instar, resulting in different sample sizes across the five instars. Consequently, the sum of the five instar-specific means does not necessarily equal the mean total larval duration calculated from the 191 complete individual records.

Morphometric Measurements

Egg diameter and height were recorded during the egg observations. Larval body length, maximum body width and head-capsule width were measured for the tracked developmental series. Larvae were measured in a natural resting position and were not stretched. Pupal length and width were measured after formation. Adult wingspan was measured after complete wing expansion as the distance between the apices of the outstretched forewings. The same anatomical landmarks and instrument orientation were used throughout the study.

Statistical Analysis

Data were summarized using sample size (n), arithmetic mean, standard deviation (SD), standard error (SE), observed range and percentages, as appropriate. Egg hatchability, stage-specific survival, pupal emergence and overall egg-to-adult survival were calculated directly from the recorded numbers of individuals entering and successfully completing each developmental stage. These proportions were treated as descriptive measures of the tracked cohort.

Developmental durations and morphometric measurements were summarized separately for each stage using the available individual records. Total larval duration was calculated from the complete individual records of larvae that successfully completed all five instars (n = 191), rather than by summing the stage-specific means obtained from unequal sample sizes.

Differences in developmental duration and morphometric characteristics among larval instars were interpreted descriptively unless supported by an appropriate inferential analysis. Because repeated measurements from the same individuals are not independent, conventional one-way analysis of variance was not assumed to be appropriate for all instar-wise comparisons.

Adult wingspan and longevity were summarized separately for males and females. Potential sex-related differences were distinguished from statistically established differences, and descriptive differences were not interpreted as significant in the absence of validated inferential results. The independence of observations, cohort structure, distributional assumptions and variance homogeneity were considered relevant to the selection and interpretation of statistical tests.

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Analyses and data verification were conducted using the individual-level developmental records and the available statistical summaries. Statistical significance was not inferred solely from differences between observed means.

RESULTS

Establishment of Laboratory Culture

The culture was established successfully from field-collected eggs and larvae. The larvae accepted fresh lemon foliage and completed all developmental stages under the laboratory conditions. Of the 360 eggs selected for individual biological observation, 163 individuals reached successful adult emergence. Losses associated with infection, parasitism, handling or developmental failure were recorded separately from normal development. The complete life cycle of Papilio demoleus is depicted in figure 1.

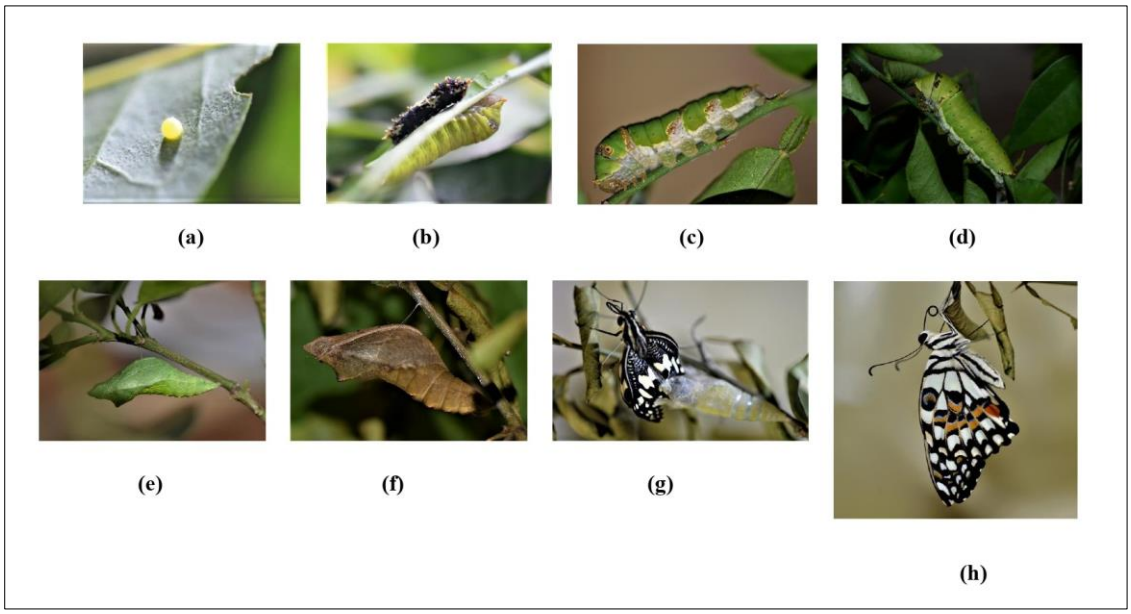


Figure 1. Life cycle and developmental stages of Papilio demoleus: (a) egg stage, (b–d) larval stages, (e–f) pupal stages, (g) adult emergence from the pupa, and (h) newly emerged adult butterfly.

Egg Stage

Fresh eggs were pale creamy yellow, nearly spherical and slightly flattened at the base. The surface was smooth and glossy. As development proceeded, the colour became duller and changed towards greyish brown, with darker streaks visible before hatching. Egg diameter ranged from 0.84 to 1.24 mm and height from 1.16 to 1.74 mm; the corresponding mean values were approximately 1.06 and 1.42 mm. Of 360 eggs followed, 276 hatched, giving 76.67% hatchability. Mean incubation period among hatched eggs was 2.84 ± 0.036 days (SE; n = 276; range 2–4 days). Eggshell consumption was observed in 233 of the 276 newly hatched larvae, while 43 larvae moved to foliage without consuming the chorion.

Larval Development

Five larval instars were recorded. Each instar was recognized from the combined pattern of body size, colour, head-capsule characteristics, feeding behaviour and moulting evidence.

First Instar

The first instar was light brown to brownish black with a dirty-white dorsal marking and small fleshy spines. The larvae remained close to tender foliage and fed mainly along the leaf margin. Movement was limited, and a short inactive period occurred before moulting.

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Second Instar

The second instar was larger and darker than the first. A broad oblique dirty-white lateral band became more distinct and the bird-dropping-like appearance was pronounced. Feeding remained concentrated on nearby leaves and the larvae became inactive shortly before moulting.

Third Instar

The third instar retained a dark-brown appearance but was clearly larger. A white dorsal marking occurred near the posterior abdominal region and feeding extended further into the leaf lamina. The osmeterium was readily everted when larvae were disturbed.

Fourth Instar

Fourth-instar larvae were almost black with a slight greenish tinge and visible whitish bands. They consumed larger areas of leaf lamina, frequently rested along the midrib between feeding periods and showed a clear quiescent phase before the next moult.

Fifth Instar

The fifth instar had a distinct mature appearance. The body became pale to yellowish green with a white sub-spiracular band, brown posterior markings and a visible yellow-to-orange osmeterium. This instar showed the greatest feeding activity. Feeding declined and wandering began before the prepupal transition.

Instar-Wise Developmental Duration

The first three instars had similar mean durations, each averaging less than three days. Mean durations were 2.78 ± 0.036 days for the first instar, 2.76 ± 0.040 days for the second and 2.83 ± 0.034 days for the third. Development increased to 3.50 ± 0.046 days in the fourth instar and 4.59 ± 0.051 days in the fifth. The fifth instar was therefore the longest larval stage. Among the 191 larvae that completed all five instars, total larval development averaged 16.57 ± 0.110 days (SE; SD = 1.53; range 13–20 days). The total larval duration was calculated from the individual sums of all five instar durations among the 191 larvae that successfully completed larval development. The mean total duration obtained from these complete records was 16.57 days. In comparison, the sum of the five separately calculated instar-specific means was 16.46 days. This difference arose because the instar-specific means were based on different numbers of successfully completing larvae (n = 252, 238, 226, 206 and 191, respectively), whereas the total larval duration was calculated exclusively from the 191 complete individual records. Thus, the reported total represents the mean of complete larval developmental periods rather than the sum of means derived from unequal samples.

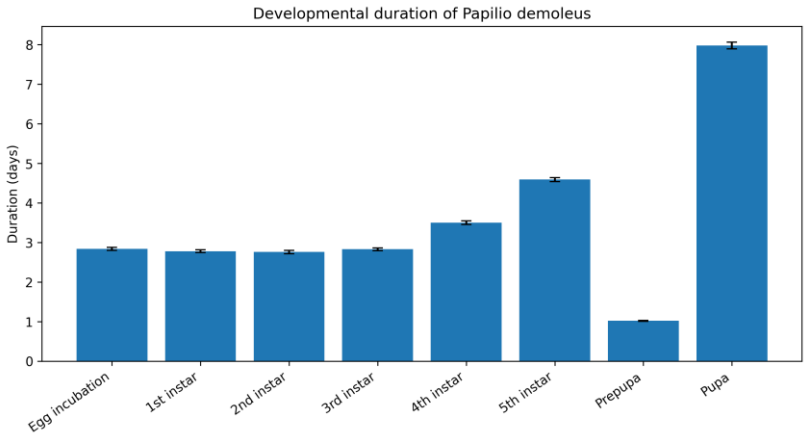


Figure 2. Mean developmental duration of egg, larval instars, prepupa and pupa of Papilio demoleus. Values are presented as mean ± SE.

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Table 1. Developmental duration of egg, larval instars, prepupa and pupa of Papilio demoleus under laboratory conditions

Stage	n	Mean ± SE (days)	SD	Range
Egg incubation	276	2.84 ± 0.036	0.59	2–4
1st instar	252	2.78 ± 0.036	0.57	2–4
2nd instar	238	2.76 ± 0.040	0.61	2–4
3rd instar	226	2.83 ± 0.034	0.52	2–4
4th instar	206	3.50 ± 0.046	0.65	2–5
5th instar	191	4.59 ± 0.051	0.71	3–7
Prepupa	187	1.02 ± 0.009	0.13	1–2
Pupa	163	7.98 ± 0.084	1.07	6–11

Instar-Wise Survival

Survival remained above 90% at every larval transition. Of 276 first-instar larvae, 252 completed the stage (91.30%). Survival was 94.44% through the second instar, 94.96% through the third, 91.15% through the fourth and 92.72% through the fifth. The prepupal transition had the highest stage-specific survival at 97.91%. Although each individual transition showed relatively high survival, the sequential losses accumulated across the complete immature period.

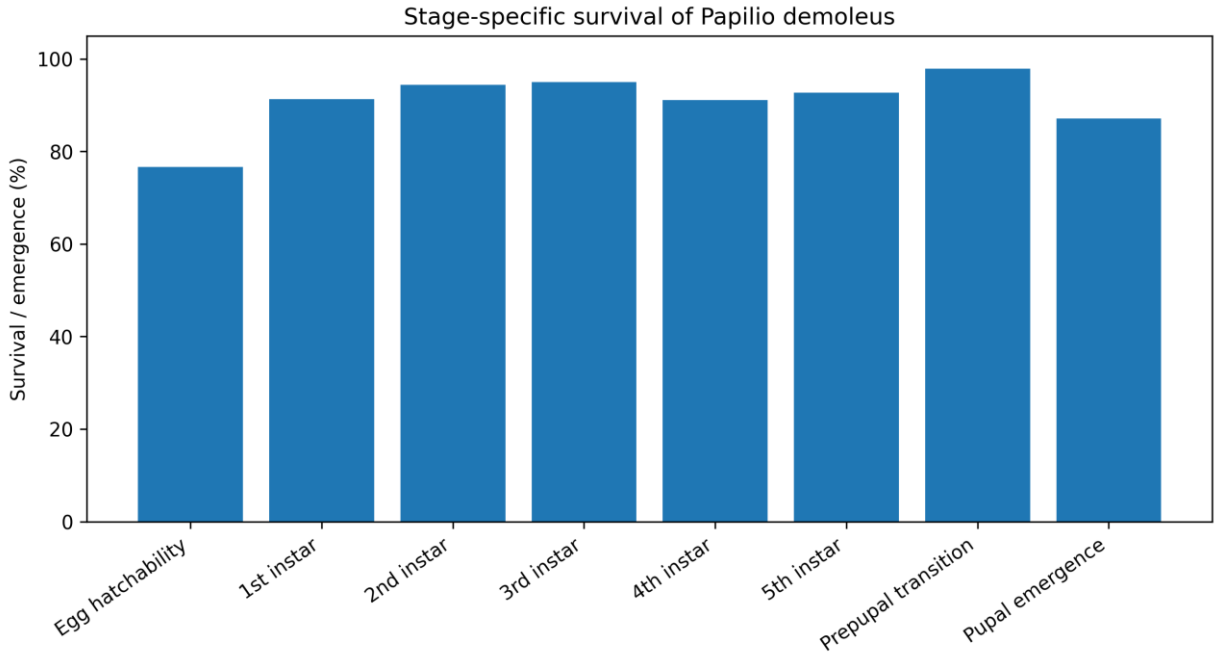


Figure 3. Stage-specific hatchability, survival and pupal emergence of Papilio demoleus under laboratory conditions. Percentages were calculated from the number of individuals successfully completing each developmental transition relative to the number entering that transition. Values represent observed proportions from the tracked cohort; error bars are not shown.

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Table 2. Developmental progression, stage-specific survival and attrition of Papilio demoleus under laboratory conditions.

Stage/transition	Entering (n)	Completed (n)	Losses (n)	Survival (%)
Egg incubation	360	276	84	76.67
First instar	276	252	24	91.30
Second instar	252	238	14	94.44
Third instar	238	226	12	94.96
Fourth instar	226	206	20	91.15
Fifth instar	206	191	15	92.72
Prepupal transition	191	187	4	97.91
Pupal emergence	187	163	24	87.17
Overall egg-to-adult	360	163	197	45.28

Stage-specific survival was calculated as the number successfully completing a developmental stage divided by the number entering that stage, multiplied by 100. Losses represent individuals that did not successfully complete the corresponding transition, including recorded mortality, developmental failure and handling-related losses where documented. The final 24 losses during the pupal-to-adult transition comprised 18 pupal deaths and six failures during eclosion or normal wing expansion. Overall egg-to-adult survival was calculated as $163/360 \times 100 = 45.28\%$.

Of the 360 eggs initially followed, 84 failed to hatch. Subsequent losses comprised 24 individuals during the first instar, 14 during the second, 12 during the third, 20 during the fourth and 15 during the fifth instar, giving 85 losses across the five larval transitions. Four additional individuals failed during the prepupal transition. Among 187 pupae, 18 died before eclosion and six failed to complete successful adult emergence or normal wing expansion. Thus, 197 of the original 360 individuals did not reach successful adult emergence, while 163 completed development successfully.

Larval Morphometric Progression

All three larval measurements increased progressively from the first to the fifth instar. Mean body length increased from 3.61 ± 0.019 mm in the first instar to 8.51 ± 0.047 mm in the second, 14.45 ± 0.050 mm in the third, 24.51 ± 0.112 mm in the fourth and 43.40 ± 0.139 mm in the fifth. This represents approximately a twelve-fold increase from the first to fifth instar. Mean body width increased from 1.55 ± 0.008 to 6.90 ± 0.022 mm over the same sequence. Head-capsule width increased from 0.65 ± 0.004 mm to 3.55 ± 0.011 mm. The largest absolute increase in body length occurred between the fourth and fifth instars.

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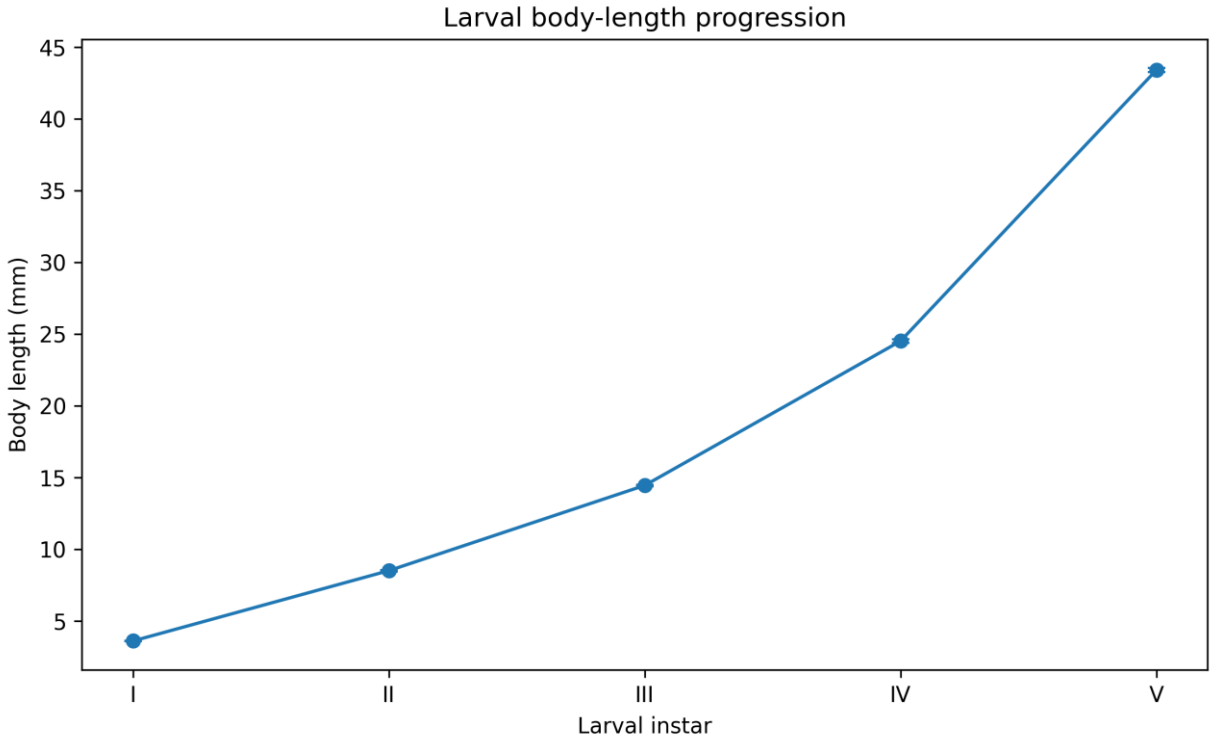


Figure 4. Progressive increase in larval body length across the five instars of Papilio demoleus. Values are presented as mean ± SE.

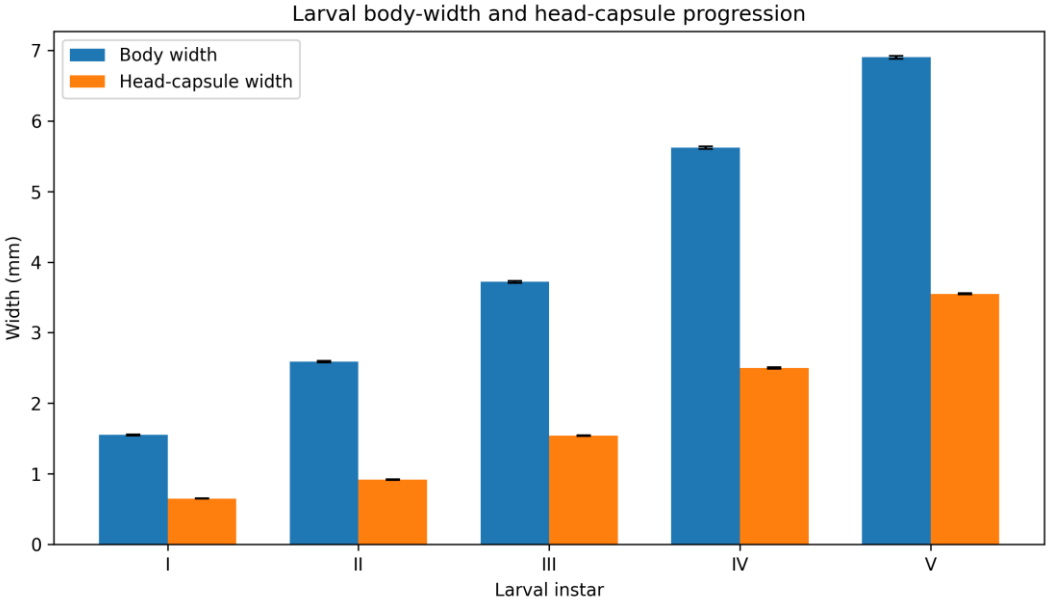


Figure 5. Progressive increase in larval body width and head-capsule width across the five instars of Papilio demoleus. Values are presented as mean ± SE.

Table 3. Instar-wise morphometric measurements of Papilio demoleus larvae

Instar	n	Length mean ± SE (mm)	Length range	Width mean ± SE (mm)	Width range	Head capsule mean ± SE (mm)	Head range
1st instar	276	3.61 ±	2.84–4.29	1.55 ±	1.10–1.90	0.65 ±	0.45–0.84

		0.019		0.008		0.004	
2nd instar	252	8.51 ± 0.047	5.92–10.36	2.59 ± 0.012	2.04–3.15	0.92 ± 0.006	0.61–1.15
3rd instar	238	14.45 ± 0.050	12.44–16.59	3.72 ± 0.016	3.02–4.37	1.54 ± 0.005	1.34–1.79
4th instar	226	24.51 ± 0.112	19.26–28.94	5.62 ± 0.018	4.67–6.31	2.50 ± 0.012	2.05–2.93
5th instar	206	43.40 ± 0.139	39.12–48.10	6.90 ± 0.022	6.01–7.64	3.55 ± 0.011	3.19–3.97

Prepupal Stage

Mature fifth-instar larvae reduced feeding, wandered away from the main feeding site, evacuated the gut and selected a stable support. Attachment was formed by a posterior silk pad and a supporting girdle. The body shortened and became curved before pupation. Mean prepupal duration was 1.02 ± 0.009 days (SE; n = 187; range 1–2 days). Four of 191 individuals entering the prepupal transition failed to complete it.

Pupal Characteristics

The pupa was angular and held to the substrate by the cremaster and silk girdle. Three colour forms were recorded among the 187 pupae: green with black speckling (93), straw or light brown (58) and dark brown with a rough appearance (36). Mean pupal length was 30.00 ± 0.088 mm (SE; SD = 1.20; n = 187; range 27.30–33.74 mm), and mean pupal width was 9.15 ± 0.016 mm (SE; SD = 0.21; n = 187; range 8.51–9.64 mm). Pupal mass was not included because the archived master workbook identifies the available weight values as allometric estimates rather than primary gravimetric observations. Among individuals that emerged successfully, pupal duration averaged 7.98 ± 0.084 days (SE; n = 163; range 6–11 days). Before emergence, the pupal case darkened and the adult wing pattern became visible through the cuticle.

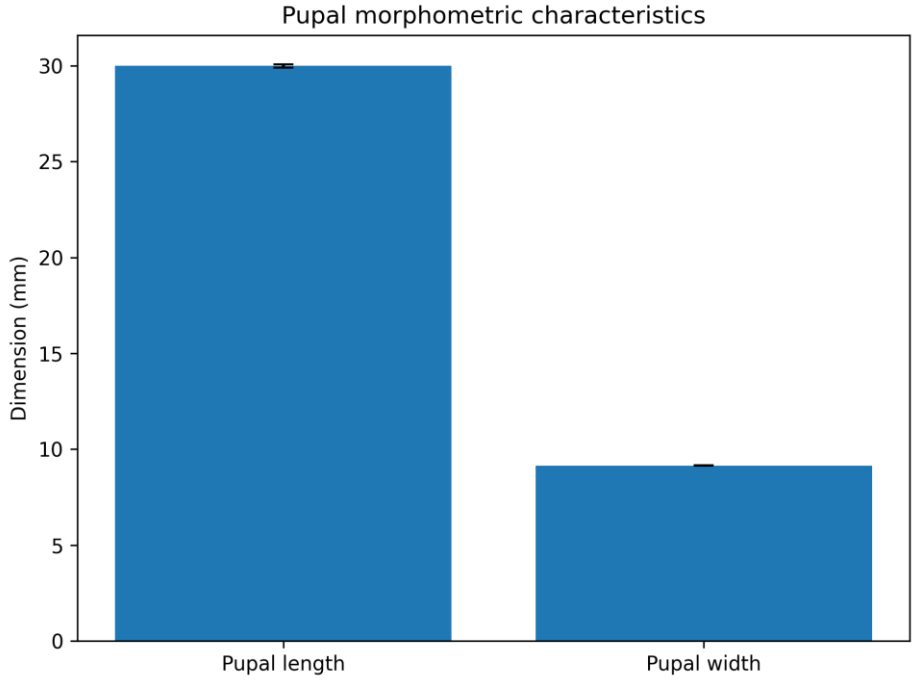


Figure 6. Mean pupal length and width of Papilio demoleus. Values are presented as mean ± SE from 187 directly measured pupae.

Table 4. Pupal morphometric characteristics of Papilio demoleus under laboratory conditions

Pupal parameter	n	Mean ± SE	SD	Range
Pupal length (mm)	187	30.00 ± 0.088	1.20	27.30–33.74
Pupal width (mm)	187	9.15 ± 0.016	0.21	8.51–9.64

Adult Emergence

Of 187 pupae, 163 adults emerged successfully, giving 87.17% pupal survival/emergence. Eighteen individuals died during the pupal period and six failed during eclosion or normal wing expansion. Newly emerged adults remained close to the pupal case while the wings expanded. Full expansion and hardening required approximately 2–3 h. The 163 successfully emerged adults comprised 69 males and 94 females, corresponding to a male:female ratio of about 1:1.36.

Adult Morphometrics

Mean wingspan was 89.90 ± 0.258 mm (SE; n = 69; range 83.22–93.99 mm) in males and 90.90 ± 0.277 mm (SE; n = 94; range 83.70–96.03 mm) in females. The observed mean wingspan was 1.00 mm greater in females than in males. This difference is presented descriptively and should not be interpreted as evidence of statistically established sexual size dimorphism without an inferential analysis that appropriately accounts for the experimental sampling structure.

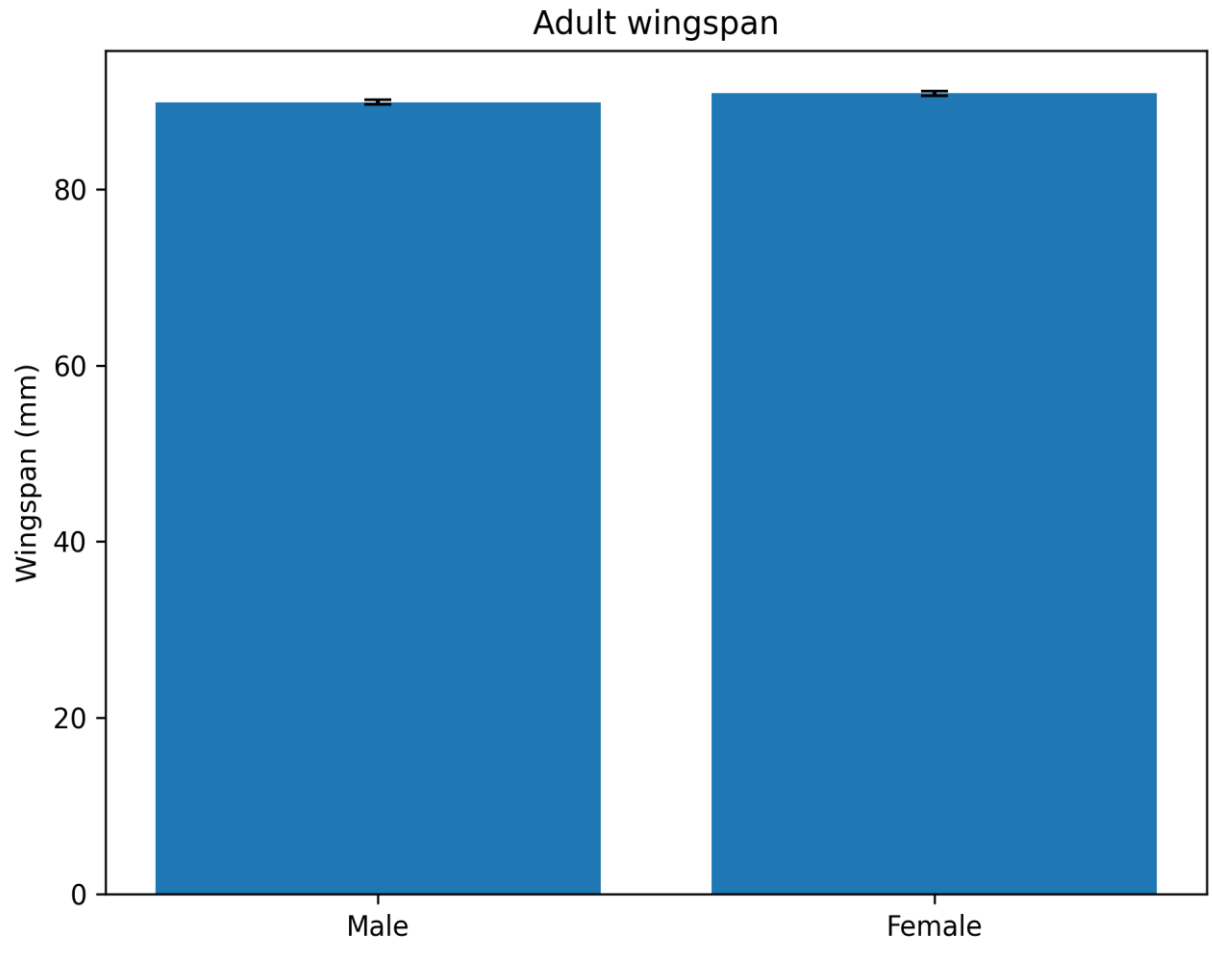


Figure 7. Mean adult wingspan of male and female Papilio demoleus. Values are presented as mean ± SE.

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Adult Longevity

Male longevity averaged 4.29 ± 0.103 days (SE; n = 69; range 3–6 days), whereas female longevity averaged 6.99 ± 0.093 days (n = 94; range 5–9 days). Female adults exhibited a mean longevity of 6.99 days, compared with 4.29 days in males, representing an observed difference of 2.70 days under the laboratory conditions. This comparison describes the observed sex-specific longevity pattern and does not independently establish statistical significance.

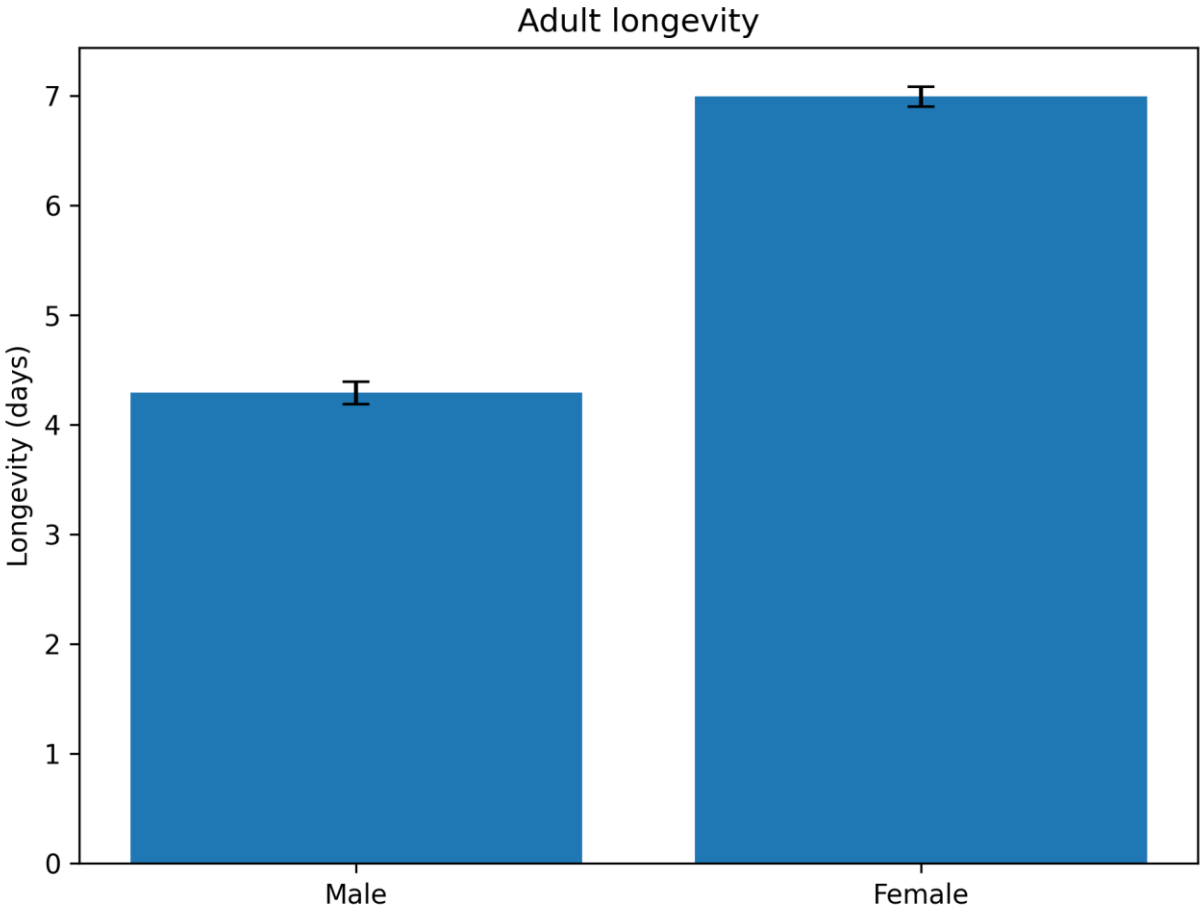


Figure 8. Mean adult longevity of male and female Papilio demoleus under laboratory conditions. Values are presented as mean ± SE.

Table 5. Sex-wise adult wingspan and longevity of Papilio demoleus

Adult parameter	n	Mean ± SE	SD	Range
Male wingspan (mm)	69	89.90 ± 0.258	2.14	83.22–93.99
Female wingspan (mm)	94	90.90 ± 0.277	2.68	83.70–96.03
Male longevity (days)	69	4.29 ± 0.103	0.86	3–6
Female longevity (days)	94	6.99 ± 0.093	0.90	5–9

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Reproductive Parameters

Mating pairs remained coupled for approximately 5–8 h. Females examined tender foliage before oviposition and laid eggs singly, mainly on tender lemon leaves and shoot tips. Reproductive measurements were available for 45 females. The pre-oviposition period averaged 1.02 ± 0.022 days, the oviposition period 4.04 ± 0.084 days and the post-oviposition period 2.00 ± 0.165 days. Mean fecundity was 106.40 ± 1.993 eggs per female, with an observed range of 71–133 eggs. Mean egg production was 26.94 ± 0.857 eggs per day.

Table 6. Reproductive parameters of female Papilio demoleus under laboratory conditions

Reproductive parameter	n	Mean ± SE	SD	Range
Pre-oviposition period (days)	45	1.02 ± 0.022	0.15	1–2
Oviposition period (days)	45	4.04 ± 0.084	0.56	3–5
Post-oviposition period (days)	45	2.00 ± 0.165	1.11	0–4
Fecundity (eggs/female)	45	106.40 ± 1.993	13.37	71–133
Eggs laid per day	45	26.94 ± 0.857	5.75	14.2–41.0

Complete Developmental Duration

Mean incubation was 2.84 days. Among the 191 larvae that completed all five instars, the total larval period averaged 16.57 ± 0.110 days (SE; SD = 1.53; range 13–20 days). The prepupal period averaged 1.02 days and the successful pupal period averaged 7.98 days. For the 163 individuals with complete linked records from egg to successful adult emergence, total immature development averaged 28.34 ± 0.169 days (SE; SD = 2.16; range 23–34 days). The same value was obtained directly from the recorded date of laying/collection to the recorded adult emergence date for these individuals. A single egg-to-death life-cycle mean was not derived by adding adult longevity to the immature mean because longevity was analysed separately.

Table 7. Duration of major developmental stages and total immature development of Papilio demoleus

Component	Mean duration (days)	Dispersion / note
Egg incubation	2.84	SE 0.036; range 2–4
Total larval period	16.57	SE 0.110; SD 1.53; n = 191; range 13–20
Prepupa	1.02	SE 0.009; range 1–2
Pupa	7.98	SE 0.084; range 6–11
Total immature period	28.34	SE 0.169; SD 2.16; n = 163; range 23–34

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The component durations were summarized using different stage-specific subsets of the tracked population. Therefore, the sum of the independently calculated component means should not be used as a substitute for the mean complete developmental duration. The total immature period of 28.34 days was calculated from the 163 individuals with complete linked records extending from egg development to successful adult emergence.

Overall Life-Cycle Survival

The tracked series declined from 360 eggs to 276 hatched larvae, 252 individuals after the first instar, 238 after the second, 226 after the third, 206 after the fourth, 191 after the fifth, 187 pupae and 163 successfully emerged adults. Overall survival from egg to adult emergence was therefore 45.28%. The decline occurred through several small losses rather than a single mortality event, with the largest proportional reduction occurring at the hatching stage and another clear loss during the pupal-to-adult transition.

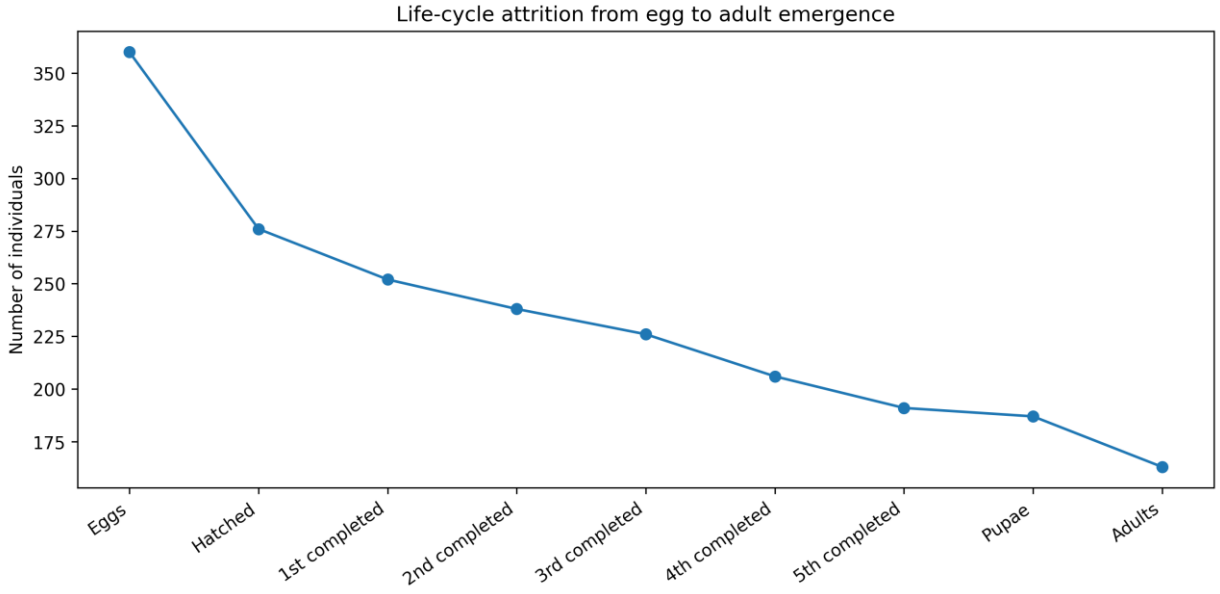


Figure 9. Developmental progression and cumulative attrition of Papilio demoleus from 360 initially tracked eggs to 163 successfully emerged adults. The figure presents the observed numbers remaining after each developmental transition. The overall egg-to-adult survival rate was 45.28%.

DISCUSSION

Egg Development and Hatchability

The incubation period recorded in the present study was short, with a mean of 2.84 days under the standardized laboratory environment. This value is close to the 2.6 days reported on lemon by Patel et al. (2017) and the 2.87 days reported on acid lime by Jahnavi et al. (2018). Dharavath et al. (2021) recorded an egg-hatching period of about 3.25 days, while longer values have been reported from other locations. Karim et al. (2007), for example, recorded an incubation period of 4.8 days, and Tien (2018) reported 5.25 days in Dalat. The difference among studies is biologically plausible because insect development responds strongly to temperature, and P. demoleus has specifically shown faster development under warmer conditions (Islam et al., 2019). The present culture was maintained at 27 ± 2 °C, so comparison with studies conducted at lower or fluctuating temperatures should be made cautiously. The present hatchability of 76.67% was obtained from a defined cohort of 360 individually tracked eggs, allowing the number of unsuccessful hatchings to be documented directly. The observation that 84 eggs did not hatch demonstrates that egg-stage losses contributed substantially to the reduction in overall developmental success. However, direct comparison of hatchability percentages between studies requires consideration of egg source, fertilization status, environmental conditions and the criteria used to classify unsuccessful development.

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Larval Development

Five larval instars were completed in the present culture. The first three were relatively short, whereas the fourth and particularly the fifth instar required more time. The total larval period of 16.57 days is almost identical to the 16.56 days reported by Patel et al. (2017) and falls close to the 17.53 days reported by Jahnavi et al. (2018). Karim et al. (2007) recorded 18.9 days, while Dharavath et al. (2021) reported a different distribution of time among individual instars. Such variation is expected when host plant, laboratory temperature, humidity, larval density and the criteria used to define moulting differ among studies. The present larval-duration estimates were verified using the recorded developmental observations. The mean total larval duration of 16.57 days was calculated from the 191 individuals completing all five instars, whereas individual instar means were calculated using different numbers of surviving larvae. This distinction explains why the sum of the separate instar means differs slightly from the complete-case total. The fifth instar required the longest mean developmental period (4.59 days), consistent with the greater body size and feeding activity observed during this stage.

Table 8. Comparison of developmental and adult biological parameters of Papilio demoleus with previous studies

Parameter	Present study	Patel et al. (2017)	Jahnavi et al. (2018)	Karim et al. (2007)
Egg incubation (days)	2.84	2.60	2.87	4.80
Total larval duration (days)	16.57	16.56	17.53	18.90
Prepupal duration (days)	1.02	—	1.04	—
Pupal duration (days)	7.98	7.16	—	—
Male longevity (days)	4.29	3.92	—	4.20
Female longevity (days)	6.99	6.80	—	6.30

The developmental measurements obtained in the present study were broadly comparable with those reported in earlier laboratory investigations, although differences were evident among populations and rearing systems. Egg incubation and total larval duration were particularly close to the values reported by Patel et al. (2017) and Jahnavi et al. (2018). Longer developmental periods reported in some other studies may reflect differences in environmental conditions, host plants, population origin and experimental procedures. Because the studies were conducted under different conditions and their individual-level datasets were not available for a common analysis, the numerical differences are interpreted descriptively rather than as statistically tested between-study effects.

Morphometric Progression

The strongest quantitative pattern was the increase in larval body size across successive instars. Mean body length increased from 3.61 mm in the first instar to 43.40 mm in the fifth, while body width and head-capsule width also increased at every moult. This pattern agrees with the general growth sequence reported for P. demoleus on citrus (Ramakrishna Rao, 2015; Patel et al., 2017; Jahnavi et al., 2018). Growth in larval insects is discontinuous because the rigid cuticle limits continuous external expansion. Substantial size increase therefore becomes evident after moulting, and head-capsule width is particularly useful for distinguishing instars because it changes stepwise rather than simply with short-term feeding state. The large absolute increase between the fourth and fifth instars also fits the biological role of the final larval stage as a period of rapid biomass accumulation before pupation.

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Survival Across Developmental Stages

Stage-specific survival exceeded 90% during every larval transition, but overall egg-to-adult survival was only 45.28%. This apparent contrast is an important feature of sequential life histories. Small losses at several stages accumulate, so high survival within individual instars does not guarantee equally high survival across the complete immature period. The largest proportional loss occurred before hatching, followed by losses at pupal emergence. Badawi (1981) also showed that mortality can vary strongly among developmental stages and generations of P. demoleus. The consolidated developmental records revealed 197 losses between the initial egg stage and successful adult emergence. Egg-stage losses accounted for 84 individuals, followed by 85 losses across the five larval instars, four during the prepupal transition and 24 during the pupal-to-adult transition. These findings demonstrate that cumulative mortality across successive developmental stages substantially reduced overall survival, despite relatively high survival percentages within individual larval instars.

The recorded loss categories included unsuccessful embryonic development, desiccation or collapse of eggs, suspected microbial involvement, parasitoid emergence, failed moulting, handling-related losses and unsuccessful adult emergence. However, the recorded classifications should not be interpreted as experimentally established causes of mortality. No controlled investigation was conducted to determine the relative causal contribution of these factors. Consequently, the survival results describe the developmental performance of the tracked laboratory cohort rather than natural mortality rates in field populations.

Prepupal and Pupal Biology

The prepupal period was brief and behaviourally distinct. Feeding stopped, larvae wandered, emptied the gut and attached to a firm support before the body shortened into the pupal form. The mean prepupal duration of 1.02 days is very close to the 1.04 days reported by Jahnavi et al. (2018). Pupal duration averaged 7.98 days among successful emergences. Mean pupal length and width were 30.00 and 9.15 mm, respectively, based on direct caliper measurements. Patel et al. (2017) reported 7.16 days, whereas longer durations have been observed in cooler environments, including the 21-day mean reported by Tien (2018). Temperature-sensitive development offers a reasonable explanation for much of this variation, but geographic population and rearing regime may also contribute (Islam et al., 2019; Kingsolver & Buckley, 2020).

Adult Emergence and Morphology

Adult emergence was successful in 87.17% of pupae. The transition from pupa to a fully functional adult included both eclosion and subsequent wing expansion, which required approximately two to three hours. Six individuals that emerged incompletely or failed to expand their wings were therefore separated from successful adults. The mean wingspan was slightly greater in females than males. Female adults had a slightly greater observed mean wingspan (90.90 mm) than males (89.90 mm). The magnitude of this difference was small relative to the mean wingspan of either sex. Previous studies have also described sex-related variation in adult morphology, although reported differences vary among populations and rearing conditions (Patel et al., 2017; Riaz et al., 2020). The present comparison is interpreted descriptively unless supported by an inferential analysis that appropriately accounts for the sampling structure.

Adult Longevity

Female adults lived longer than males under the same feeding regime. Mean longevity was 6.99 days for females and 4.29 days for males. Patel et al. (2017) reported corresponding values of 6.80 and 3.92 days, and Karim et al. (2007) recorded 6.3 and 4.2 days. Jahnavi et al. (2018) also reported longer female than male longevity. The consistency of this pattern across several laboratory studies suggests that sex-specific adult life history is a regular feature of the species, although longevity remains sensitive to food supply and environmental conditions. Dharavath et al. (2021) showed that adult diet can noticeably alter recorded lifespan, which is relevant when comparing studies using different sugar or honey solutions.

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The observed difference of 2.70 days between female and male longevity was substantial relative to the mean lifespan of males. Nevertheless, adult longevity can be influenced by nutritional conditions, mating activity, cohort characteristics and laboratory maintenance procedures. The present findings therefore describe sex-specific longevity under the experimental conditions and should not be generalized directly to natural populations.

Reproductive Parameters

The observed females had a short pre-oviposition period and laid eggs over several days. Mean fecundity was 106.40 eggs per female, considerably higher than the means reported by Munir et al. (2007), Karim et al. (2007) and Tien (2018), but close to the 112 eggs reported by Ramakrishna Rao (2015). This wide variation among studies indicates that fecundity is particularly sensitive to rearing context. Adult nutrition, mating success, female size, larval host quality, observation period and whether all eggs deposited in the cage are recovered can affect the final estimate. The current reproductive dataset was based on 45 observed females and should therefore be interpreted as performance under the present laboratory system rather than a fixed species-wide fecundity value.

Novel Contribution and Biological Significance

The principal contribution of the present investigation is the integrated assessment of developmental duration, stage-specific survival, larval morphometric progression, pupal characteristics, adult longevity and reproductive performance within a tracked laboratory culture of Papilio demoleus originating from central India. Although these biological characteristics have been investigated individually in earlier studies, the present work provides a linked developmental account extending from 360 eggs to 163 successfully emerged adults.

The study demonstrates that relatively high survival during individual larval transitions does not necessarily result in high overall developmental success. While survival exceeded 90% during each larval transition, cumulative egg-to-adult survival was 45.28%. The complete-case calculation of larval duration and the documentation of developmental losses also provide a transparent basis for comparing laboratory populations.

The morphometric observations further establish a progressive increase in body length, body width and head-capsule width across successive larval instars. The fifth instar showed the greatest mean body length and longest mean developmental duration, consistent with its role as the final feeding and growth stage before pupation.

Together, these observations provide a locally derived laboratory reference for developmental studies of P. demoleus on Citrus limon. The results may assist in larval stage identification, experimental culture maintenance and the design of subsequent investigations into host-plant effects and environmental influences. However, the study did not directly quantify field damage or test management interventions, and its applied implications should therefore be regarded as potential applications rather than experimentally demonstrated pest-control outcomes.

STUDY LIMITATIONS

The findings of the present study should be interpreted in relation to several methodological limitations.

First, the investigation was conducted under controlled laboratory conditions of 27 ± 2 °C, 70 ± 5% relative humidity and a 14 h light:10 h dark photoperiod. Although these conditions supported standardized developmental observations, they did not reproduce natural fluctuations in temperature, humidity, rainfall, solar exposure and host-plant availability. Consequently, the developmental durations and survival percentages observed under laboratory conditions may differ from those occurring in natural populations.

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Second, the experimental culture was established using field-derived material collected from the Government Holkar Science College campus in Indore, Madhya Pradesh. Although multiple cohorts were maintained, the study represented a geographically restricted source population. Geographic variation, local adaptation and differences among genetically distinct populations were not investigated. Therefore, the findings should not be generalized to all populations of Papilio demoleus without additional comparative studies.

Third, larvae were maintained exclusively on fresh Citrus limon foliage. Host-plant species, leaf maturity, nutritional composition and plant defensive compounds may influence larval growth, survival and developmental duration. Because alternative citrus hosts were not compared within this experiment, the extent to which the observed biological parameters depend on the selected host plant remains uncertain.

Fourth, no parallel field validation was conducted. Natural populations experience predation, parasitism, variable food quality, climatic fluctuations and other ecological pressures that are not fully represented under laboratory rearing conditions. In addition, handling procedures, container density, sanitation practices and artificial adult feeding may have influenced developmental success and adult longevity. The reported survival percentages therefore describe outcomes under the experimental rearing system rather than natural population survival.

Fifth, reproductive observations were available for 45 females, representing a subset of the successfully emerged female adults. Reproductive measurements may therefore not fully represent variation across the entire experimental population.

Finally, the interpretation of statistical comparisons was limited by the availability of validated inferential results and the need to account for repeated measurements and potential clustering within rearing cohorts or containers. Descriptive differences were not treated as statistically significant without appropriate analytical support.

Future investigations should include geographically distinct populations, comparisons among citrus host plants, environmentally variable rearing conditions and field-based validation to establish the general applicability of the developmental patterns reported here.

CONCLUSION

The present study documented the developmental biology, survival and morphometric progression of Papilio demoleus under standardized laboratory conditions using Citrus limon as the larval host plant. Of 360 individually tracked eggs, 276 hatched successfully and 163 individuals completed development to adulthood, representing 76.67% hatchability and 45.28% overall egg-to-adult survival.

Five larval instars were recorded, with mean durations of 2.78, 2.76, 2.83, 3.50 and 4.59 days, respectively. The mean total larval duration was 16.57 days, calculated from the complete individual records of 191 larvae successfully completing all five instars. The fifth instar represented the longest larval developmental stage and exhibited the greatest body dimensions. Stage-specific survival exceeded 90% during each larval transition, but cumulative losses across successive developmental stages reduced overall survival to less than half of the initial egg cohort. Larval body length increased from 3.61 mm in the first instar to 43.40 mm in the fifth, accompanied by progressive increases in body width and head-capsule width. Mean prepupal and pupal durations were 1.02 and 7.98 days, respectively. Female adults showed greater observed mean longevity than males, and reproductive observations from 45 females indicated a mean fecundity of 106.40 eggs per female.

The integrated developmental records provide a quantitative laboratory reference for P. demoleus originating from central India. Comparisons with previous studies indicate broad similarities in developmental patterns, alongside differences that may be associated with environmental conditions, host plants and population origin.

Because the present investigation was restricted to laboratory conditions and a single geographic source population, further studies involving alternative hosts and field validation are required before extending these findings to natural populations.

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