

Research Article

Biology of the Endemic Endangered Swallowtail Butterfly, *Papilio desmondi teita* (Lepidoptera: Papilionidae), on Wild Citrus Species in Taita Hills, Kenya

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The Taita Hills swallowtail butterfly, *Papilio desmondi teita*, was reared in captivity on wild citrus (Rutaceae), *Toddalia asiatica* and *Clausena anisata*, in Ngangao Forest, Taita Hills, Kenya. The butterflies were exposed to the host plants for oviposition inside the laying buckets under ambient laboratory conditions. Results revealed that *P. desmondi teita* could only complete development on *T. asiatica* and but not on *C. anisata* in both seasons. The egg incubation period on *T. asiatica* was significantly shorter (P < 0.001) compared to *C. anisata* during the first season (P = 0.595) but not in the second season. The development period of the butterfly in 1st and 2nd seasons on *T. asiatica* was 81.13 ± 0.44 days and 112.15 ± 1.20 days, respectively, while a female-biased sex ratio was observed in the first and second seasons. Findings from this study indicate that *T. asiatica* was the most suitable host plant for development of *P. desmondi teita*.

1. Introduction

Butterflies are among most significant component of biodiversity within the ecosystem [1]. Some butterflies such as papilionids and pierids and some groups of nymphalids and hesperiids are pollinators of wild flowering plants and cultivated crops [2]. Approximately 87.5% of flowering plant species depend on animal pollinators for their production [3]. In the agricultural sector, 87 of the leading world food crops and 35% of the total crop production volumes are dependent on animal pollination [4]. Most butterflies are typically herbivores in their larval stages and majority are host specific with a close relationship with their host plants [5]. Also, most adults have a specialized mouthpart, the proboscis which is long and tubular for extraction of nectar, a primary source of nutrition for adult butterflies [6, 7]. While feeding on plants' nectar, butterflies play a critical role as pollinators through the transfer of pollen from one flower to another [6]. However, some butterfly species particularly in the family Nymphalidae

feed on other liquid substances including fruit juice, rotting fruit, tree sap, and even animal droppings [6–8].

Swallowtail butterfly species are endangered and have been experiencing a reduction in diversity, occurrence, and abundance across the globe [9-11]. The loss of pollinator swallowtail butterflies is linked with the reduction in plants that they depend on through habitat change and destruction activities such as deforestation, agriculture conversion and intensification, alteration of pastures, and urbanization [12]. These alterations within their natural habitat have a negative impact on their fundamental life functions such as mating, breeding, foraging, and many others [13]. This has raised concerns about the stability of ecosystem function and food security [14]. These factors may also cause a local extinction or migration of the endemic swallowtail butterflies due to the loss of their preferred host plants within their habitats [15, 16]. Among the tropical swallowtail butterfly species, Papilio desmondi teita (Van Someren) (Lepidoptera: Papilionidae) is an endemic and endangered species to Taita Hills in Kenya [9, 17] that form the Eastern Arc Mountains (EAM) [7, 12, 18].

The knowledge of the relationship between swallowtail butterflies and their host plants is very scanty. According to a recent study, compared to farmlands, forest edges are home to a greater diversity of butterfly species [19]. A crucial step in achieving the conservation goal of swallowtail butterflies is through understanding their biology within their natural habitats. Once biology is understood, a more sustainable management and conservation strategy could be designed and adopted for use. Currently, there is no known information on the biology of P. desmondi teita with very little information documented on its larval host plants by Larsen [20] and Congdon et al. [21]. Therefore, the present study was conducted to document the biology of the swallowtail butterfly P. desmondi teita on wild species of citrus (Rutaceae), horsewood Clausena anisata (Willd) and orange climber Toddalia asiatica (Lam), which were previously reported as the species' larval host plants according to Larsen [20] and Congdon et al. [21] as well as to understand the larval host preference between the selected host plants.

Clausena anisata, a wild citrus species, is a small, deciduous shrub with imparipinnate compound leaves that are heavily gland-dotted and have a strong, anise-like scent when crushed [22]. According to Mukandiwa et al. [23], the shrub is the only member of the Clausena genus found in tropical Africa [24]. Its country-specific geographical range includes bushveld, riverine thickets, and forests throughout sub-Saharan Africa, with the exception of the driest regions. Toddalia asiatica, a woody liana that is a wild citrus species, can grow up to 10 meters high in the forest [25]. According to Gopal et al. [26], it is extensively distributed throughout Madagascar, Asia, and Africa's tropical regions. In the Maasai and Kipsigis communities of East Africa, the plant is traditionally used as a hedge and as a browser for goats [27]. The host plants in this study were specifically chosen as the larval host plants due to their frequent occurrence in the Taita Hills and their utility to the local butterfly farmers during the reconnaissance survey. This study will yield valuable information for the broader conservation of swallowtail butterflies and the plants that serve as their hosts in Kenya and throughout sub-Saharan Africa.

2. Materials and Methods

2.1. The Study Site. Taita Hills which form part of Eastern Afromontane Biodiversity Hotspot [7, 28, 29] with an elevation range of 1,200 to 2,200 meters above sea level are home to an exceptionally high number of endemic plant and animal species (Figure 1). The study was conducted at the Dawida Biodiversity Center, which is situated in the coastal region of Wundanyi Subcounty, Taita Taveta County, Kenya, at the Ngangao Forest fragment of the Taita Hills.

The study was carried out at two locations within the study site: a garden (a) and a laboratory (b) between March and September of 2021. In a garden under shade, oviposition was observed in buckets holding the mated females and the two host plants, *C. anisata* and *T. asiatica*. The garden recorded ambient temperature and relative humidity of 23.87 ± 0.93 °C and $66.83 \pm 3.22\%$, respectively, during the

first season (March–May 2021) and an ambient temperature and relative humidity of $18.25 \pm 0.57^{\circ}$ C and 74.83 ± 1.51 %, respectively, in the second season (June–September 2021). Subsequent studies on development from egg (collected from oviposition experiment) to adult were conducted inside the laboratory at an ambient room temperature and relative humidity of $22.19 \pm 0.09^{\circ}$ C and $73.86 \pm 0.33^{\circ}$, respectively, during the first season (March–May 2021) and ambient room temperature and relative humidity of $18.26 \pm 0.13^{\circ}$ C and $74.71 \pm 0.50^{\circ}$, respectively, during the second season (June–September 2021). All the experiments were carried out at 12L:12D in both seasons.

2.2. Host Plants. Clausena anisata (Willd.) Hook. f. ex Benth.—Habit: Shrub. Habitat: Forest margins and regenerations, bushland, wooded grassland, 1700–1875 m. Vouchers: SAJIT–004600, Watuma BM W0069 (EA, HIB), Mwachala G et al. EW012, Faden RB et al. NMK Exped. 263 (EA).

Toddalia asiatica (L.) Lam., Tabl. Encycl. 2: 116 1797. (syn. Aralia labordei H. Lév.; Cranzia aculeata (Sm.) Oken; Cranzia asiatica (L.) Kuntze; Cranzia nitida Kuntze; Limonia oligandra Dalzell (Unresolved); Paullinia asiatica L.; Rubentia angustifolia Bojer ex Steud. (Unresolved); Scopolia aculeata Sm.; Scopolia angustifolia Spreng. (Unresolved); T. aculeata (Sm.) Pers.; T. angustifolia Lam.; T. asiatica var. floribunda (Wall.) Kurz; T. asiatica var. gracilis Gamble; T. asiatica var. obtusifolia Gamble; T. floribunda Wall.; T. nitida Lam.; T. rubricaulis Roem. & Schult.; T. tonkinensis Guillaumin; Zanthoxylum floribundum Wall. (Unresolved).

Two wild citrus species, Clausena anisata (Willd.) and Toddalia asiatica (L.) Lam. (Rutaceae) were chosen as the host plants for the experiment on swallowtail butterfly, *P. desmondi teita* developmental attributes. From the forest edges, young branches with new leaves from each host plant were gathered and brought to the laboratory. After washing the branches to get rid of dust and any potential predators, they were submerged in water to prevent desiccation. The branches were placed in plastic buckets (325 mm dia. \times 375 mm·h) labeled with the host plant species for use in colony maintenance.

2.3. Swallowtail Butterfly, Papilio desmondi teita, Culture. A colony of P. desmondi teita from eggs (collected from an oviposition experiment), larval instars, pupae, and adults was maintained inside the laboratory (situated in Ngangao Forest) in plastic buckets (Figure 2(b)) on C. anisata and T. asiatica. The stock colony was established from the adults collected at the forest edges in November 2020. The adults were collected using a sweep net while foraging on the flowers from various plant species, and some females were collected on the selected host plants while laying eggs. To avoid rubbing off the scales on the upper surface of the wings, the butterflies' wings were folded above their backs before being placed in waxed paper envelopes. After being brought to the laboratory, they were placed in rearing flight cages that were 70 cm by 70 cm by 70 cm in size. A mosquito net was placed over the cages to provide ventilation. They



FIGURE 1: Map illustrating study site at Ngangao Forest, Taita Hills (developed using ArcGIS, ILRI database, 2018) (source: Genga et al. [18]).

were also kept from escaping by the nets. Twice a day, they were given 10% sugar solution soaked in cotton wool as food. To ensure successful mating, the males and females were artificially mated after 24 hours using the hand pairing method [18, 30]. Mated females were individually placed into laying buckets containing either young branches of the host plant, *C. anisata* or *T. asiatica*, for oviposition.

From the oviposition experiment, all eggs laid on the same day and at the same age on each host plant were collected and placed into clearly labeled transparent plastic



FIGURE 2: Egg-laying site outside the laboratory at a garden (a) and laboratory rearing in the laboratory (b) within Ngangao Forest, Taita Hills.

buckets that measured 325 mm in diameter by 375 mm in height. These buckets were then used for incubation and further development in the laboratory. Throughout both seasons, the P. desmondi teita rearing cultures were kept at room temperature and relative humidity. The mesh-covered, detachable lids on the buckets improved air circulation. After hatching, the young larvae were placed in clean rearing plastic buckets (325 mm Dia × 375 mm·H) with young, fresh host plants inside, and they were separated from the unhatched eggs using a soft camel-hair brush. The young, fresh leaves of the selected plants were fed to the larvae.

The pupae were removed after they had pupated by gently pulling them off and scratching the corner of the silk girdle that the cremaster was attached to. The pupae were moved to the new buckets, and twigs and white serviette paper were added to the base to support the emerging adults. Up until their adult emergence, the pupae were kept in the same laboratory conditions. After a day, the newly emerged adults were fed with a 10% sugar solution and separated from the pupae. By using the hand pairing method, six pairs were chosen from the

population that was reared from each host plant, and they were mated after two days [30]. Within the rearing bucket, the mated females were exposed to their host plants in preparation for oviposition. The second season experiment followed the same procedure used during the first season.

2.4. Effects of Host Plants on Egg Incubation Period and Mortality Rate. After being separated from the other adults, six mated females were put in buckets with young, fresh branches from each host plant in preparation for oviposition. Eggs of the same age (laid on the same day) were kept in the buckets as mentioned in the previous section, with their lids turned upside down for ventilation, to determine the egg incubation period on each host plant. Until they hatched, the eggs were watched every day. The incubation period was determined by counting the days until the eggs hatched. To determine the egg mortality rate, the number of unhatched eggs from each host plant was counted. Mortality was calculated as a percentage.

Percentage egg mortality = $\frac{nu}{to}$	$\frac{\text{mber of unhatched eggs}}{\text{otal number of laid egg}} \times 100. $ (1)
2.5. Effect of Host Plants on Larval Development Period,	size, marking and pattern, and head capsule coloration. The
Mortality Rate, and Larval Weight. In plastic rearing buckets	larval weight was determined by weighing 60 individual larvae
with adequate ventilation and a branch of young, fresh leaves	using JA-SERIES (JA203) electronic analytical balance
from the host plants, a cohort of 165 larvae (in the first season)	(0.001 mg accuracy). By monitoring larval cohorts at each instar
and 192 larvae (in the second season) was placed. The duration	and keeping track of the number of survivors from the start of

larval mortality rate =
$$\frac{\text{number of dead larvae at the end of instar}}{\text{total number of larvae at the beginning of instar}} \times 100.$$
 (2)

2.6. Effect of Host Plant on Pupal Period, Pupal Weight, and Mortality Rate. Pupae were marked with the date of formation, and those of the same age were kept together in the

of larval instar was determined by recording the number of days

taken to complete each instar stage identified by differences in

rearing bucket and observed daily. Sixty pupae reared on each host plant were used to determine pupal development parameters such as pupal period and pupal weight. During

each instar to the finish, the mortality rate was determined. The

mortality rate (by instar) was calculated with the formula

the experiment, the pupal mortality rate was also recorded. The time taken from pupal formation to the adult butterfly emergence was recorded in days as the pupal period. An electronic analytical balance (Type JA203H; JA-SERIES) with a 0.001 mg accuracy was used to weigh each pupa. To calculate the pupal mortality rate, the number of dead pupae (those that did not fully transform into adults) were counted and recorded. Abbott's [31] formula was used to calculate the mortality rate:

$$pupal mortality rate = \frac{number of emerged adults}{total number of pupae formed} \times 100.$$
(3)

2.7. Effect of Host Plant on Adult Longevity, Sex Ratio, Weight, and Oviposition. By examining the morphology of the forewing, hindwing, and abdomen, adult butterflies were sexed. The sex ratio was calculated by counting and recording the number of adult males and females. The adult longevity of males and females fed on 10% sugar solution was investigated by recording the time from each individual emergence to death on a daily basis. The number of eggs laid in the first two days was used to calculate the oviposition. After a day, at 6 pm, when they were less active, sixty newly emerged adults were weighed individually on an electronic analytical balance (0.001 mg accuracy; Type JA203H; JA-SERIES). All experiments were replicated three times and in two seasons.

2.8. Statistical Analysis. All the data were analyzed in R statistical software package, version 4.1.1 [32]. Mean and SEM (standard error of mean) were computed for the duration of developmental stages, egg incubation period, oviposition, egg mortality rate, larval and pupal mortality, and weight of developmental stages. The independent sample *t*-test was used to compare the means from each host plant. The Shapiro–Wilk test and the Bartlett test of homogeneity of variance were applied to the fecundity data on various substrates and submitted to one-way analysis of variance (ANOVA) using general linear model (GLM) procedure of *R* statistical package software. Tukey's multiple comparison test was used to separate the means between the substrates. $P \le 0.05$ was designated as the significance level.

3. Results

3.1. Egg Incubation Period and Mortality. The females of *P. desmondi teita* laid eggs singly on the branch, lower and upper sides of the leaves, and bucket. They laid significantly more eggs on the lower leaf surface ($F_{3,76} = 50.19$; P < 0.001) compared to other oviposition substrates when exposed to *T. asiatica* while on *C. anisata*, most eggs were laid on the bucket surface followed by the lower leaf surface and the least was laid on the stem surface as shown in Table 1. In the first season, the host plants significantly influenced

TABLE 1: Oviposition preference and mean $(\pm SE)$ number of eggs laid by *Papilio desmondi teita* on *Clausena anisata* and *Toddalia asiatica* (as per plant) in March 2021 under field conditions.

Oviposition substrate	<i>Clausena anisata</i> Number of eggs	<i>Toddalia asiatica</i> Number of eggs
Upper leaf surface	$0.75 \pm 0.39 bc$	$7.10 \pm 1.36b$
Lower leaf surface	$3.65 \pm 0.70b$	$25.60 \pm 2.63a$
Stem	$0.20 \pm 0.16c$	$2.60 \pm 0.61 b$
Bucket	$8.80 \pm 1.39a$	$2.95 \pm 0.55b$
F _{3,76}	23.82	50.19
P value	< 0.001	< 0.001

Means (±SE) in the same column followed by the same letter do not differ significantly at $P \le 0.05$ (Tukey's test).

(t = 16.15; df = 188; P < 0.001) the incubation period with the eggs laid on *C. anisata* hatching in 8.35 days compared to 7.27 days for those laid on *T. asiatica*. However, during the second season, the host plants did not significantly influence the incubation period at t = -0.53; df = 182; P = 0.595 for eggs laid on *C. anisata* and *T. asiatica* host plants. The percentage mortality for the eggs laid on both host plants did not differ significantly at t = -0.14; df = 4; P = 0.895 and t = 0.00; df = 4; P = 0.998 in both rearing seasons as outlined in Table 2.

3.2. Larval Development and Mortality Rate. Swallowtail butterfly, *P. desmondi teita*, larvae recorded five instars in both seasons. Immediately upon hatching, the first instar larvae were feeding on their empty eggshells before moving toward the host plant that was provided. The larvae feed on *T. asiatica* successfully completed the five instar stages of development. Those reared on *C. anisata* died between 2 and 3 days in both seasons. The larval development period on *T. asiatica* varied from the 1st to 5th instar larvae in both seasons. The longest period was recorded in fifth instar stage for both seasons, and the shortest period was recorded in 1st and 2nd instar in 1st and 2nd seasons, respectively (Table 3). The total larval period for the larvae reared on *T. asiatica* was 44.55 \pm 0.27 days in the first season and 66.05 \pm 0.66 days in the second season.

The larval instar mortality rate observed on *T. asiatica* during the first season varied from 8.51% for first instar to no mortality for the 5th, with 24.34% mortality for all larvae reared on *T. asiatica*. Similarly, the mortality rate observed on *T. asiatica* in the second season varied across the instar stages with the highest rate recorded in the first instar and lowest rate in the fifth instar. The overall mortality rate for the larvae reared on *T. asiatica* during the second season was 32.61%. However, the larvae that were reared on *C. anisata* recorded 100% mortality in the first instar, hence leading to the end of the experiment in both seasons. Therefore, the overall larval mortality recorded in both seasons for the larvae reared on *C. anisata* was 100% (Table 3).

The host plants significantly influenced the weight of the first instar larvae at t = 3.637; df = 188; P = 0.0004 during the first season. The first instar larvae reared on *C. asiatica* were heavier $(1.42 \pm 0.03 \text{ mg})$ than those reared on *T. asiatica* $(1.29 \pm 0.02 \text{ mg})$. However, the first instar larvae reared on

	Egg development parameters/seasons			
Host plant	March–May	2021	June–Septemb	ber 2021
	Incubation period (days)	Mortality rate (%)	Incubation period (days)	Mortality rate (%)
Clausena anisata	$8.35 \pm 0.05a$	$36.38 \pm 17.07a$	$11.33 \pm 0.05a$	$16.82 \pm 3.73a$
Toddalia asiatica	$7.27 \pm 0.04 b$	$39.55 \pm 14.64a$	$11.37 \pm 0.05a$	$16.81 \pm 2.85a$
Т	t = 16.15	-0.14	-0.53	0.00
P value	<0.001	0.895	0.595	0.998

Means (\pm SE) in the same column followed by the same letters do not differ significantly at $P \le 0.05$ (t-test).

TABLE 3: Mean (\pm SE) of larval instar duration (days), total larval period (days), and larval instar specific percentage mortality of *Papilio desmondi teita* reared on *Clausena anisata* and *Toddalia asiatica* during the first season (March–May 2021) and second season (June–September 2021) under laboratory conditions.

T	6		Host p	olants	
Larval instar	Season	N	T. asiatica	N	C. anisata
1 st	1^{st}	96	6.00 ± 0.00	85	_
1	2 nd	78	10.77 ± 0.08	—	_
and	1^{st}	95	6.29 ± 0.05	—	_
2	2 nd	77	8.97 ± 0.09	—	_
ard	1^{st}	95	7.92 ± 0.03	—	—
5	2 nd	75	11.40 ± 0.13	—	—
⊿ th	1^{st}	77	7.44 ± 0.09	—	—
4	2 nd	70	12.77 ± 0.16	—	—
∈ th	1 st	77	16.90 ± 0.10	—	—
3	2 nd	65	22.14 ± 0.20	—	—
Total lawsal mania d	1^{st}		44.55 ± 0.27		_
iotai iarvai period	2 nd		66.05 ± 0.66		—
I amral in stan	Casaan		Mortality (%)/host plant	
Larvai instar	Season	Ν	T. asiatica	N	C. anisata
1 st	1 st	96	8.51 ± 1.01	85	100 ± 0.00
1	2 nd	78	19.04 ± 5.13	_	100 ± 0.00
and	1 st	95	0.88 ± 0.88	_	_
2	2 nd	77	3.75 ± 2.14	—	_
ard	1 st	95	1.88 ± 0.94	—	_
3	2 nd	75	1.33 ± 1.33	—	_
4 th	1^{st}	77	6.38 ± 4.53	—	_
4	2 nd	70	6.72 ± 2.64	—	_
⊊th	1 st	77	0.00 ± 0.00	_	_
0	2 nd	65	12.41 ± 7.96	_	_
Lamral montality	1 st		24.34 ± 9.09		100 ± 0.00
Larvar mortanty	2 nd		32.61 ± 3.53		100 ± 0.00

N=number of larvae cohort studied; - indicates no record of numbers/no observation.

C. anisata died between 24 and 36 hours while those reared on *T. asiatica* completed all the instar stages recording mean weights of 5.42 ± 0.23 mg, 28.05 ± 1.40 mg, 85.12 ± 3.20 mg, and 493.64 ± 18.04 mg in second, third, fourth, and fifth instars, respectively. Similarly, during the second season, the host plants significantly influenced the weight of first instar larvae at t = -3.92; df = 182; P = 0.0001. The first instar larvae reared on *T. asiatica* were heavier $(1.35 \pm 0.03 \text{ mg})$ than those reared on *C. anisata* (1.21 ± 0.02 mg). All the first instar larvae reared on *C. anisata* died within 36 hours. On the contrary, the larvae reared on *T. asiatica* successfully completed all the larval stages recording mean weights of 6.62 ± 0.14 mg, 28.91 ± 0.36 mg, 113 ± 1.90 mg, and 420.56 ± 7.99 mg in the 2^{nd} , 3^{rd} , 4^{th} , and 5^{th} instars, respectively.

3.3. Pupae Development, Mortality, and Total Development Period (Egg-Adult). The pupae of *P. desmondi teita* reared on *T. asiatica* took 27.31 \pm 0.13 days in the first season and 31.73 \pm 0.49 days in the second season for adults to emerge. The mortality rate of pupae reared on *T. asiatica* was 7.88 \pm 2.48% during 1st season and 4.11 \pm 2.41% in the 2nd season. The mean pupal weight of the pupae reared on *T. asiatica* during the first season was 1165.55 \pm 17.17 mg while the second season recorded a mean weight of 1102.41 \pm 22.58 mg.

3.4. Adult Longevity, Oviposition Preference, and Sex Ratio in *P. desmondi teita*. The females' longevity was 9.08 ± 0.10 days, which was significantly longer (t = 14.19; df = 69; P < 0.001) than that for males at 7.23 ± 0.08 days in the first season. A

similar trend was recorded for the second season during which females recorded 6.89 ± 0.08 days and males 5.12 ± 0.11 days at t = 12.69, df = 68, P < 0.001. Overall, the development time from egg to adult ranged between 81.13 days in the first season and 112.15 days in the second season both reared on *T. asiatica* (Table 4). The female to male sex ratio was 1:0.97 and 1:0.95 during the first and second season, respectively.

During the first season, females of *P. desmondi teita* reared on *T. asiatica* had a mean weight of 481.47 ± 11.85 mg which was significantly higher than 300.80 ± 8.51 mg recorded for males (Table 4), and the same trend was recorded for the second season. The number of eggs laid by the females of *P*. desmondi teita on *T.* asiatica and *C.* anisata did not differ significantly within the first two days. (t = 0.65; df = 4; P = 0.550) during the first season.. However, during second season, the host plant species significantly influenced the number of eggs laid by the butterflies at t = -2.94; df = 4; P = 0.042. The mean number of eggs laid was 42.00 on *T. asiatica* and 35.00 on *C. anisata*.

4. Discussion

The host plants selected in this study were found to influence the development and oviposition of P. desmondi teita. The study showed that the female laid eggs singly on the upper and lower sides of the fresh leaf, bucket surface, and branch surface. The highest number of eggs was laid on the lower leaf surface of T. asiatica while on C. anisata, it was observed that most eggs were laid on the bucket surface. The higher number of eggs laid on the lower side of T. asiatica leaf was meant to protect the eggs from being washed away by rainfall, direct sunlight, and natural enemies including predators and parasitoids [33]. This observation was consistent with what Stamp [34] reported about the majority of papilionid butterfly species. However, the observation made when P. desmondi teita was exposed to C. anisata for oviposition could be attributed to the contact chemical stimuli on the host plants which plays a great role in female final decision to lay eggs [35], which in the present case, the females were trying to avoid this host plant which was unsuitable for their larval survival as exhibited from this findings. The same observation about egg-laying habits was also made in a study on P. demoleus and P. polytes in Bangladesh by Islam et al. [36, 37]. The habit of laying a single egg prevents the possibility of larval feeding resources being depleted, allowing for the efficient use of host plant resources [38]. Higher nutrient accumulation and smooth textures lead to the oviposition observed on the host plants' young, fresh leaves [33].

The egg incubation period for *P. desmondi teita* eggs varied on each host plant. Generally, eggs laid on *T. asiatica* had a shorter incubation period as compared to those on *C. anisata*. The variation in incubation period might be accounted for by the different host plants due to the variation of accumulated secondary plant semiochemicals [39]. Findings by Reddy and Guerrero [40], Hardie et al. [41], and Kahuthia-Gathu [42] confirmed that plants' semiochemicals play a major role in female oviposition preference to host plant choice. The incubation period might also vary due to

fluctuations in temperature during the period of study as observed in our study. The first season in March 2021 recorded the highest mean temperature compared to the second season in June 2021. These results are similar to the finding of Al-Mehmmady [43] who reported incubation period of *Earias vittella* (Fabricius) at 2.42 days during September at 31.3°C and 2.15 days during August at 32.6°C. Similarly, Syed et al. [44] reported the shortest incubation period of *E. vittella* during September when the average laboratory temperature was 32.6°C and the longest incubation period during October with an average temperature of 30.6°C.

In this study, no significant differences were registered in the percentage egg mortality between the host plants for P. desmondi teita in both seasons. These results could probably be attributed to the female oviposition behaviour as a determinant of successful reproduction [45]. Similarly, females have the capability to invest in the survival of their progeny by inducing mechanisms such as secretion of sticky substances that protect the eggs against parasitism, endowing the eggs with substances that deter predation, and increasing the egg size [46, 47]. However, the percentage mortality recorded during the study could be associated with host toxins and infection, environmental factors in the two seasons, and host contact with xenobiotic factors beyond control during the experiment [48]. However, we could not justify whether the mortality rate registered on the eggs was a result of the host plant response factors or due to the natural causes within the environment, thus creating the need for further investigation on the actual causes of death.

The developmental periods and larval mortality varied considerably within the two seasons. The observed variation could be associated with biotic and abiotic factors such as differences in nutrition components and semiochemicals released from the host plants and changes in environmental conditions experienced during development [49]. This study revealed that *P. desmondi teita* larvae could only complete all successive larval instar developmental stages on T. asiatica and were unsuccessful on C. anisata. This observation could be related to unsuitability of C. anisata as a host plant for P. desmondi teita [50] which led to the death of first instar larvae as a result of starvation. Congdon et al. [21] reported a similar observation that T. asiatica was host plant for P. desmondi. The present study however did not confirm the report by Larsen [20] that *Clausena* species was the host plant for the butterfly species. 100% mortality was recorded in the first instar larvae reared on C. anisata in both seasons in the present study. In addition, the first instar larvae of P. desmondi teita were observed avoiding the C. anisata host plant. The 100% mortality on C. anisata could have been a result of larvae starvation due to avoidance of the host plant. This could be attributed to the biochemical compounds present in C. anisata that might be repellant to first larval instar of P. desmondi teita. This conforms to the findings of Visser [51] who reported that herbivorous insects have developed adaptive mechanisms to identify suitable hosts and evade nonsuitable hosts using their scents. The study also showed that young larvae had a greater mortality rate than older stages. On the Anaphe panda (Boisduval)

TABLE 4: Mean (\pm SE) adult weight, fecundity, and de(June-September 2021) under field conditions (for JMarch-hHost plantMaleFemaleweight (mg)Weight (mg)T. asiatica300.80 \pm 8.51B481.47 \pm 11.85A12.33	velopment fecundity) May 2021 P value <0.001	time to adult of and laboratory Oviposition rate (number of eggs per female) $44.00 \pm 5.20a$	f Papilio desmo conditions (fo Development time to adult (days) 81.13 ± 0.44	ndi teita reared or 1 development tii pment parameters Male weight (mg) 292.58 ± 11.34B	n Toddalia asiati ne to adult). s Female weight (mg) 444.76±6.19A	a in the first June-Septe T P valu 12.12 <0.00	season (March-May 202 mber 2021 Oviposition rate (number of eggs per female) 1 42.00 ± 1.15a	1) and second season Development time to adult (days) 112.15 ± 1.20
C. anisata T P value		0.550 ± 0.550	I	I	I		0.042 ± 0.050 -2.94 0.042	
Means (\pm SE) followed by the same lower case letters in th	ne same coli	umn and same uj	pper case letters	in the same row do	not differ signific	antly at $P \leq 0$.)5 (<i>t</i> -test).	

Aay 2021) and second season	
utica in the first season (March-	
<i>iondi teita</i> reared on <i>Toddalia asic</i>	for development time to adult).
nt time to adult of <i>Papilio desm</i>	r) and laboratory conditions (f)
ight, fecundity, and developme	field conditions (for fecundity
.E 4: Mean (±SE) adult wei	e-September 2021) under

(Lepidoptera: Thaumetopoeidae) at Kakamega Forest in Kenya, Mbahin et al. [52] reported similar findings. In the present study, *P. desmondi teita* registered higher mortality in first instar and fifth instar stages as compared to other stages of larval development on *T. asiatica*. The factors that might have likely contributed to mortality of the first instar larvae were unknown; however, the mortality registered on fifth instar larvae was likely caused by sharp internode spines present on the stem and lower surface of the leaves of *T. asiatica* [53]. These findings are consistent with those reported on the survival of silkworm, *A. panda*, in Kakamega Forest of Western Kenya by Mbahin et al. [52].

Duration of development can prolong when the amount of food consumed is reduced, and this results in the insect's reduced length and weight [54] as on P. desmondi teita for the larvae reared during the two seasons on T. asiatica. When P. desmondi teita was reared on T. asiatica, the fifth instar's larval duration was longer than the first, second, third, and fourth instars. The fifth instar also recorded the highest weight which was a result of the longer feeding duration registered during the study while compensating for the low feed intake observed in the previous instars. Carvalho and Vasconcello-Neto [55] found a similar observation about the larval performance and host plant selection in Mechanitis polymnia casabranca (L.), a species of neotropical butterfly. Similar findings on P. polytes, a distinct species within the same family reared on citrus plants, were documented by Suwarno et al. [56]. The results on *P. desmondi teita* showed that fifth instar larvae had the highest weight which differed greatly from the weight of fourth instar larvae in both seasons. This could be attributed to increasing feeding at the fifth instar stage and prolonged feeding duration as reported by Hochuli [57] and Tithi et al. [58].

The pupal period of P. desmondi teita reared on T. asiatica varied within the seasons, ranging from 27.31 days to 31.73 days in the 1st and 2nd seasons, respectively. Variable pupal period and total development period could be a result of differences in climatic conditions between the seasons. Furthermore, the nutritional stage attained during larval development typically determines pupation after the larval phase [42]. Once the fifth instar larva has accumulated sufficient reserve for successful pupation and adult emergence, pupation takes place. The reserve accumulation is greatly influenced by the host plants [59]. Findings reported by Al-Mehmmady [43] showed that the pupal period of E. vittella during August and October were 6.45 and 7.78 days with an average temperature of 32.6°C and 30.5°C, respectively. Furthermore, the duration of the development period increases with a decrease in temperature as reported by Syed et al. [44] on Earias vittella when reared on okra, Abelmoschus esculentus L., China rose, Hibiscus rosasinensis L., cotton, Gossypium hirsutum L., and Indian mallow, Abutilon indicum L. They conducted their study of the three life cycles of E. vittella, keeping the temperature regime at a difference of 2°C between different life cycles in a laboratory and recorded an increase in the duration of the life cycle when the temperature decreased in July.

Up to 80% of the food provided to the larva during its fifth instar is devoured by it; hence, its growth increases in direct proportion to the amount of food it consumes. If the

amount of available food is limited, this could result in small pupa with low weight even if the diet is nutritionally sufficient [60]. This could be the case with P. desmondi teita reared on *T. asiatica* which had a variation in pupal weight in the first and second seasons. The lowest mortality rate was observed at the pupal stage of development as compared to the larval stages in *P. desmondi teita*. These results are similar to the findings reported by Kahuthia-Gathu et al. [42] on Plutella xylostella (L.) (Lepidoptera: Plutellidae) reared on cultivated and wild crucifer species. Islam et al. [37] reported a mortality rate of 7.69% on the same genus P. polytes reared on citrus species in the laboratory which was within the range reported in the present study. Halloran and Wason [61] reported the highest mortality during the egg stage. Additionally, Suwarno [62] found the highest mortality at the fifth instar. The variations reported in mortality rates from previous findings and the current study may be associated with weather changes, human error, and host plant effects. These findings are indications that the adoption of bucket rearing technology has great potential to increase the survival of the species to adults which could contribute to its conservation and management. Also, the technology could be adopted for commercial butterfly farming for the species used for trade.

In the present study of P. desmondi teita, the highest adult longevity was recorded in the first season and the lowest in the second season. Females generally lived longer than males. The higher longevity in females could be related to the need of finding mates as described by Pereira et al. [63]. Similar findings were reported by Al-Mehmmady [43] on E. vittella male and female longevity reared during August and October in Saudi Arabia. However, the present study was in contrast with Syed et al. [44] who reported that males of E. vittella within the same order Lepidoptera lived longer than females. Jahnavi et al. [64] reported that females of Papilio demoleus (L.) (Lepidoptera: Papilionidae) lived longer than the males when reared on acid lime in Tirupati. Females were generally heavier than the males in our study. Mackey [65] and Lederhouse et al. [66] reported that females achieve their greater size than males because of their larvae in various larval stages which feed more and develop for a longer period than the larval stages of the males. A previous study by Scriber and Slansky [67] observed that Lepidoptera females often weigh more than males over the majority of their life cycle, a characteristic that has been linked to the function of laying eggs. Swallowtail butterfly, P. desmondi teita, had a female-biased sex ratio in the present study. This finding showed that T. asiatica was a suitable host plant for the mass production of the endangered swallowtail butterfly species endemic to Taita Hills.

The result from the present study showed variation in the effect of the host plants on female fecundity of *P. desmondi teita* exposed to both *C. anisata* and *T. asiatica*. Generally, more eggs were laid on *T. asiatica* compared to those laid on *C. anisata*. The finding of this study was clear evidence that *P. desmondi teita* larvae reared on *T. asiatica* produced females that showed generally a better performance on *T. asiatica* than on *C. anisata*, a clear demonstration that *T. asiatica* was a more profitable host plant for *P. desmondi*

teita female oviposition. Kahuthia-Gathu et al. [42] observed that adult P. xylostella reared on wild crucifers produced females that performed better than those reared on cultivated crucifers. Following a previous study that demonstrated that most female butterflies will make higher oviposition within the first two days after mating [18], this study explored oviposition on both host plants within the first two days after mating. Similarly, David and Gardiner [68] discovered that females have a greater egg load at this age following mating. Findings reported on the same genius P. demoleus by Patel et al. [69] showed that the fecundity ranged at 110.80 ± 4.46 eggs per female on citrus. Similarly, Maheswarababu [70] reported almost similar fecundity of 112 eggs per female P. demoleus on citrus species. In the present study, the fecundity ranged between 35.00 to 53.33 eggs per female P. desmondi teita were recorded on the target host plants, which was lower than those reported above by Patel et al. [69] and Maheswarababu [70] on P. demoleus in the same genus.

The findings of this study revealed that *P. desmondi teita* could only complete development on *T. asiatica* and was unsuccessful on *C. anisata.* This was clear evidence that *T. asiatica* was the most suitable host plant for the species, and thus the study recommends the conservation of *T. asiatica* within its natural habitat for the survival of this species assessed as an endangered species according to IUCN [9] species red list assessment. With this knowledge, more sustainable conservation strategies for this endangered swallowtail butterfly species may be designed accordingly. Furthermore, policymakers and other stakeholders should use this study's findings as a starting point when developing policies aimed at achieving long-term conservation goals for this butterfly species and its natural environment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The financial source was not involved in the development of the study design, data collection and analysis, publication decision, or manuscript writing.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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