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Research Article

Biology of the Wild Silkmoth Anaphe panda (Boisduval) in the Kakamega Forest of Western Kenya

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A study on the life cycle of the silkmoth *Anaphe panda* (Boisduval) was conducted in two different habitats of the Kakamega Forest in western Kenya: Ikuywa, an indigenous forest, and Isecheno, a mixed indigenous forest. Eggs were laid in clusters, and the incubation period ranged from 40 to 45 days. Larvae fed on *Bridelia micrantha* (Hochst) and passed through seven instars. The developmental period took between 83 to 86 days in the dry season and 112 to118 days in the rainy season. The pupal period ranged between 158 and 178 days in the rainy season and, on the other hand, between 107 and 138 days in the dry season. But the later caught up in development with those that formed earlier. Moths emerged from mid-October until mid-May. Longevity of adult *Anaphe panda* moths took between 4 and 6 days, but generally females seemed to live longer than males. The moth also seems to have higher lifespan in the indigenous forest compared to the mixed indigenous forest.

1. Introduction

Wild silk farming is a supplementary activity for income generation for rural communities that mainly depend on subsistence farming and assist in conserving the wild silkmoth and its habitat. Anaphe panda Boisduval (Lepidoptera: Thaumetopoeidae) is one of the indigenous silkmoth species in the Kakamega Forest that is currently used in silk production by the community surrounding the Kakamega Forest. The huge nest of Anaphe pupal cocoons can be degummed to produce brown silk that is of high quality. Although aspects on the conservation of this wild silkmoth for economic incentives to rural communities [1, 2], its spatial distribution [3, 4], and the use of sleeve nets to improve survival of the Boisduval silkworm [5] are known, their biology and life cycle have not been studied. Biological information is essential to formulate suitable management strategies for the sustainable utilization of these wild silkmoth resources [6]. At this crucial time of climate change, the objective of the present study was to gather more information on the development and annual life cycle and seasonal occurrence of A. panda silkmoth which is one of the treasures of people living around the Kakamega Forest of western Kenya.

2. Materials and Methods

2.1. Study Area. Four different types of forests are found in the Kakamega Forest: forest with only indigenous species (indigenous forest), mixed indigenous forest (forest with indigenous and exotic species), hardwood (forest with exotic hard species only), and softwood plantations (forest with exotic soft species only). The study was conducted in two different habitats of the Kakamega Forest, Isecheno and Ikuywa at Lunyu and Ikuywa sublocations, Ileho Division, in the Kakamega Forest, western Kenya. The Kakamega Forest is located between latitudes $0^{\circ} 10'$ and $0^{\circ} 21'$ North and longitudes 34° 47' and 34° 58' East (Figure 1). The entire Kakamega Forest covers a total area of approximately 265 km². It comprises several separate blocks of forest of which Isecheno (415 ha) belongs to Lunyu sublocation and Ikuywa (380 ha) belongs to Ikuywa sublocation. Sampling was carried out during a period of three years (2005–2007) in two project sites: Chirobani (mixed indigenous forest) and Musembe village (indigenous forest) (Figure 1). The natural vegetation of Kakamega Forest is tropical rainforest. Apart from the indigenous forest and mixed indigenous forest, the area has a variety of other habitats including hardwood



FIGURE 1: Study sites in the Kakamega Forest of Western Kenya.

and softwood plantations. There are about 150 species of woody trees, 90 species of dicotyledonous herbs, 80 species of monocotyledonous herbs of which about 60 are orchids, and a further 62 species of ferns totalling to about 380 identified species of vascular plants [7].

2.2. Egg Cluster Characteristics and Incubation Period. To know the egg size, one hundred eggs obtained from twenty-five *A. panda* female moth were measured using a stereomicroscope with an ocular micrometer. A Vernier calliper was used to determine the average length of eighty-three freshly laid egg clusters. Their weight with golden brown hairs was determined by electronic balance Mettler PJ 360 DeltaRange. One hundred and ninety-five egg clusters which were laid daily were marked to determine the exact incubation period. Daily observations were made on hatching for thirty-five days.

2.3. Larval Period. The larval period was determined by observing from the 1st to 7th instars 180 cohorts of *A. panda* larvae on *B. micrantha* until they span. The observed number was 120 and 78 in the dry season and rainy season, respectively. The rearing was done by protecting the entire larval stage of eighty-six (86) cohorts with net sleeves, and another ninety-four (94) cohorts were unprotected. The number of instars was determined by visual observation of exuviae [8, 9]. Earlier field observations showed that younger larvae rest about two days on the leaf, and older ones (from three to four days) attach themselves on the bark of *B. micrantha* before moulting. Hence, observations were made daily at 10:00 am at that period to observe if the larvae cast their skin (exuviae). The total number of instars.

2.4. Pupal Period. To determine pupal period, spinning date of 100 cocoon nests was marked and kept in the enclosed net sleeve $(60 \times 40 \times 30 \text{ cm})$ in the field until moths emerged.

2.5. Adults. Time of emergence was determined by direct observation and also by the marked pupae which were kept on *B. micrantha* in the net sleeve in the field. The life span of male moths was also recorded for comparison with that of the female moths. Wing expanse was determined by measuring with Vernier calliper, the maximum distance between the tips of the forewing after spreading the moths. This was the measurement from the apex of the forewing and its attachment to the thorax.

2.6. Environmental Data. A digital hygrothermometer (Zheda Electric Apparatus Inc., http://www.zjlab.com/) was used for recording daily temperature (maximum and minimum), and measurements of relative humidity were recorded four times daily (6 am, 12 am, 3 pm, and 9 pm) at both sites throughout the period of the study. A rain gauge was used for recording rainfall data.

2.7. Data Analysis. A *t*-test and chi-square [10] was used for all the means comparisons. The means of the analysis were compared between the two blocks of forest using the Stata7 software [11]. The degree of significance was indicated conventionally as follows:

*: significant (*P* < 0.05), **: highly significant (*P* < 0.001), ns: nonsignificant (*P* > 0.05).

3. Results

3.1. Egg Cluster. The eggs of A. panda are usually laid in clusters (Figure 2(d)) underside of leaves of the host plant B. micrantha (Figure 6). The mean number of freshly laid eggs in a cluster was 395.167 ± 40.736 and 485.313 ± 89.9 in the mixed indigenous (n = 54) and in the indigenous forest (n = 49), respectively. Highly significant difference was found to exist between the mean numbers of eggs in the two habitats. B. micrantha was the only observed host plant for A. panda





FIGURE 2: Anaphe panda silkmoth life stages: (a) male; (b) female moths; (c) mating; (d) egg cluster; (e) hatching (1st instar silkworms); (f) 7th instar silkworm on the host plant *Bridelia micrantha*; (g) Cocoon nest; (h) Pupae in the cocoon nest.



FIGURE 3: Developmental velocity of *Anaphe panda* silkworm in the Kakamega Forest.

oviposition in the Kakamega Forest. The eggs are small, and they are usually discoidal and isodiametric measuring 0.963 ± 0.008 mm as a major axis and 0.61 ± 0.004 mm as a minor axis. The mean length and weight of the egg cluster (n = 40) was 1.64 ± 0.16 cm and 0.22 ± 0.04 g in the mixed indigenous forest, whereas in the indigenous forest (n = 43)it was 1.66 ± 0.2 cm and 0.25 ± 0.07 g for the length and weight, respectively. No significant difference was observed between the length and the weight of the freshly laid egg cluster of the two habitats.

The incubation period ranged from 40 to 45 days in the dry season and from 45 to 55 days in the rainy season. Mean incubation periods at the two habitats are summarized in Table 1.

In the same habitat, high significant difference was found to exist between the dry season and rainy season. No significant difference was found between the two habitats.

3.2. Larvae. The larvae are gregarious (Figure 2(e)) from the first to the seventh instars. The direct observation of cast exuviae by the larvae reared through the entire larval stage showed that six moults occurred from egg hatch to the pupation. The larval stage was between 83 and 86 days in the dry season and 112 and 118 days in the rainy season.

From the egg hatches, young caterpillars follow prodigious growth from 3 mg to more than 3,000 mg (Figure 2(f)). The developmental velocity in the whole larval stage is shown in Figure 3.

A highly significant difference was found to exist between the dry season and rainy season of the larval period in the same habitat. There was no significant difference in larval period between the two habitats.

3.3. Pupa. When larvae were fully grown (Figure 2(f)), they started to spin silk. The pupa (Figure 2(h)) was enclosed in a tough silk cocoon (Figure 2(g)). In rainy season, the duration of the pupal period ranged between 158 and 178 days, and between 107 and 138 days for those which spun in dry season. Mean pupal stage duration by habitats is summarized in Table 2.

In the same habitat, highly significant difference was found to exist between the dry season and the rainy season. No significant difference was found between the two habitats. Furthermore, no significant difference was found to exist between the three years of observation of this study.

	Isecheno block (mixed indigenous forest)				Ikuywa block (indigenous forest)			
Years	1st brood		2nd brood		1st brood		2nd brood	
	п	Incubation	п	Incubation	n	Incubation	п	Incubation
2005	12	41.75 ± 1.82	8	49.88 ± 3.91**	13	41.69 ± 1.65	10	$49.9 \pm 3.57^{**}$
2006	17	42.82 ± 1.91	18	$50.78 \pm 3.69^{**}$	16	42.19 ± 1.97	15	$49.87 \pm 3.58^{**}$
2007	16	42.13 ± 1.86	18	$50.39 \pm 3.15^{**}$	18	41.83 ± 1.79	17	$50.77 \pm 3.54^{**}$

TABLE 1: Means (±SD) incubation period in days of Anaphe panda in the Indigenous and mixed indigenous forest in Kakamega.

n: number of egg clusters; **: highly significant difference between the 1st and 2nd broods.

Voors	Duood	Isecheno	(mixed indigenous forest)	Ikuywa (indigenous forest)		
lears	brood	n	Pupa period (days)	п	Pupa period (days)	
2005	1	6	167.5 ± 7.52**	9	167 ± 7.7	
2005	2	7	122.14 ± 10.49	8	8 126.25 ± 11.88	
2006	1	10	$168 \pm 6.06^{**}$	8	168 ± 7.86	
2000	2	9	123.33 ± 12.94	6	121.5 ± 5.24	
2007	1	9	166.67 ± 5.43**	8	169 ± 8.33	
	2	9	122.33 ± 10.69	11	124.64 ± 11.94	

TABLE 2: Mean $(\pm SD)$ pupal period (days) of *A. panda* in the Kakamega Forest.

n: number of cocoon nests; **: difference highly significant between the 1st and the 2nd broods.

The length and width of male ranged from 1.8 to 2.7 cm (mean 2.11 \pm 0.17) and 0.6 to 1 cm (mean 0.73 \pm 0.07), respectively, while the length and width of female pupae were from 2.1 to 3.1 (mean 2.4 \pm 0.15) and 0.6 to 1.3 cm (mean 0.82 \pm 0.08), respectively. High significant differences (*P* < 0.001) were found to exist between the lengths and also the widths of the two sexes. The weight of the male pupal ranged from 0.23 to 0.9 g (mean 0.5 \pm 0.14), whereas female pupae weight ranged from 0.27 to 1.16 g (mean 0.73 \pm 0.19). There were also significant differences between the weights of the pupae of the two sexes.

3.4. Adults. Moths (Figures 2(a) and 2(b)) were found in the forest from October to April. The peak numbers were observed in late November to late February (Figure 4). The mean wingspan of forewing was 2.347 ± 0.216 cm and 3.019 ± 0.251 cm for males (n = 2701) and for the females (n = 3088), respectively, whereas the mean lengths of forewing was 1.137 ± 0.106 cm and 1.448 ± 0.138 cm for males and females, respectively.

There was a high significant difference between the wingspan and the mean length of forewing of the male and the female moths. *A. panda* mean life span in Kakamega Forest is summarized in Table 3.

Time of occurrence and developmental periods of various stages of *A. panda* in the Kakamega Forest is summarized in Figures 2 and 4, respectively.

3.5. Environmental Data. The environmental data on mean monthly average temperature, humidity, and rainfall for the two locations were reported in Figures 5(a) and 5(b), respectively. Note that rainfall was bimodal in the Kakamega Forest, with a period of long rains from April through June, and short rains in August through November. During the

study, the annual rainfall ranged from 180.9 to 265.9 cm at Isecheno and from 188.6 to 224.7 cm at Ikuywa. The annual number of rainy days ranged from 196 to 219 and from 207 to 209 in Isecheno and Ikuywa, respectively. Mean monthly maximum temperature ranged from 15.5°C to 36.8° C at Isecheno, and from 16.5 to 35.6° C at Ikuywa. Mean monthly maximum humidity ranged from 45.4 to 86.2% in the mixed indigenous forest and from 35.6 to 80.9% in the indigenous forest.

4. Discussion

Observations in this study showed that one egg cluster had 350 to 539 eggs, contrary to Jolly et al. [12] and Kioko et al. [1] who noted one cluster of 250-350 and 250-300 eggs, respectively. Depending on the health of the female, the number of eggs laid may vary from just a few to one hundred [13]. The number of eggs per cluster does not vary significantly (P > 0.05) for different generations in the same habitat (block of forest). Nevertheless, a highly significant difference (P < 0.001) was observed between the indigenous and the mixed indigenous forest. The incubation period depends on climatic conditions. Results obtained from this study confirm an earlier study by Jolly et al. [12] who recorded that the embryonic period may last a month or more (45 days) depending on climatic conditions. The fact that there was no significant difference between the two habitats means that temperature may have strongly influenced the incubation period.

The time between hatching from the egg and the completion of larval stages varied. This depends upon the availability of food, a favourable climate, and other factors [13]. The total larval period of *A. panda* is 120 days. This differed from the 140 days reported by Jolly et al.

TABLE 3: Adult lifespan	(in days) of A.	panda from indigenous and	mixed indigenous forest ir	1 Kakamega (vears 2005–2007)
	(

Years		Isecheno block (ndigenous forest)		Ikuywa block (indigenous forest)					
	п	Male	п	Female	<i>t</i> -test	n	Male	n	Female	<i>t</i> -test
2005	51	4.545 ± 1.036	51	5.182 ± 0.982	ns	50	5 ± 0.943	60	6.25 ± 0.639	**
2006	65	3.88 ± 1.013	51	5.091 ± 0.701	**	60	5.25 ± 1.209	64	6.25 ± 1.032	*
2007	71	4.323 ± 1.043	56	5 ± 0.632	*	57	5.235 ± 1.251	79	6.333 ± 0.701	**

ns: non significant difference; *: significant difference between male and female; **: highly significant difference between male and female; *n*: Number of males or females moths.



FIGURE 4: Time of occurrence of the various stages of Anaphe panda in the Kakamega Forest.



FIGURE 5: (a) Rainfall, temperature, and relative humidity in the mixed indigenous forest (Isecheno). (b) Rainfall, temperature, and relative humidity in the indigenous forest (Ikuywa).

[12] for *A. venata.* The general relationship between the developmental velocity, expressed in terms of weight and the number of days tends to be nonlinear (Figure 3); it appears to be exponential. This finding is similar to that of Wigglesworth [14]. From the results obtained in these experiments, it appears that the duration of the larval instars is dependent upon temperature. High temperatures during dry season cause a shorter duration, while low temperatures during rainy season prolongs the development. This agrees with observations made in studies both by Geertsema [15] and Chapman [16]. According to Wigglesworth [14],

temperatures and nutrition may also have an effect on the number of larval instars.

In this study, a delay of emergence of moths in the larvae was observed in the dry season. Pupal diapause which extended over five months was also recorded in this study. This probably explains the significant difference in the records between the two seasons. Adult moths of the brood of the dry season emerge after 168 days, and the brood of rainy season emergence was after 122 days. This indicates that the rate of development of the pupae differs between the two seasons and is most probably influenced by intrinsic



FIGURE 6: *A. panda* host plant *Bridelia micrantha* with egg cluster in the Kakamega Forest.

factors. Van den Berg's [17] study on the *N. cytherea clarki* had similar conclusion that pupae formed during the later (warmer) season were able to catch up in development with those formed earlier in the colder season.

A. panda longevity in days of male and female does not differ significantly (P = 0.324 > 0.05) for different generations. But difference between the two blocks of the forest was highly significant (P < 0.001). Generally, moths seem to have higher life span in the indigenous forest compared to the mixed indigenous forest. There was also a highly significant difference between the two sexes in both blocks of the forest (P < 0.001). Females live longer because they perhaps must lay the maximum number of eggs before dying. Observations made in this study confirm earlier findings by Jolly et al. [12] who reported that an adult *Anaphe* sp. lives for nearly a week.

Highly significant differences (P < 0.001) were observed between the means of the forewing expanse and the length of females and males. Mean forewing expanse and length of females were larger than those of the males. The size of male and female seems to be one of the differences in this insect.

According to this study, February and March are the best months to harvest dry cocoon nests with minimal damage to fresh cocoons when moths are not emerged. To develop wild silkmoth farming, life cycle information is very important. By understanding the life cycle, the species and its food plants can be conserved and the community helped to utilize it for income generation.

The climatic conditions observed in this study (Figures 5(a) and 5(b)) were consistent with reports by Muriuki and Tsingalia [18] and Kokwaro [19]. As poikilothermic organisms, the life cycle, activity, distribution, and abundance of Lepidoptera are influenced by temperature [20]. Pollard and Yates [21] found that temperature and rainfall were likely to influence the survival of butterflies directly and indirectly through the effects on plant growth, disease, predation, or other factors. In light of the present study, further work is warranted to understand why a forest insect like *A. panda* periodically develops high populations in certain well-defined types of forest habitat, but not in all habitats where it occurs.

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