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Three-year lifecycle, large body, and very high threshold temperature in the cricket *Gryllus argenteus* for special adaptation to desiccation cycle in Malawi

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Abstract In temperate climates, the initiation and termination of diapause synchronize the stress-tolerant stage with the stressful season and reproduction with the non-stressful season in many insects. Synchronization is often regulated by photoperiodism.

Voltinism and the ultimate size of adults are also important determinants for their lifecycle, and different diapause stages and voltinism patterns are known in crickets.

Here, we investigated the life history of the African cricket *Gryllus argenteus* from Malawi, which is a typical arid tropical highland. The climate is characterized by alternating arid and wet seasons, each of which lasts for half a year, and where the available heat mass is much less than lowlands at the same latitude. We first measured the nymphal duration at each rearing temperature and calculated the lower developmental threshold (t_0) to be 20.19 °C based on Ikemoto and Takai (2000) and 19.38 °C based on a conventional line-fitting method. These values are very high relative to many other insects. The local temperature in winter does not fall below 15 °C, but this is much higher than the lethal limit. This suggested that critical stress in this locality was not coldness but low precipitation in winter. We estimated, based both on local temperature change and the Ikemoto and Takai's t_0, that *G. argenteus* required 3 years to complete its lifecycle unlike wet lowland species, where univoltinism or multi-voltinism are commonplace. Photoperiodism was observed in this species, but due to a lag between annual cycles in photoperiod, temperature, and humidity, photoperiodism alone cannot atune their lifecycle with local conditions.

Synchronization in this species was achieved by three different adaptations: photoperiodism, high t_0, and large body size, which give it a long lifecycle. Although the species cannot achieve a univoltine lifecycle because of its high t_0 value, it can escape from dry season by entering diapause at moderate temperatures, probably thereby achieving adaptive synchrony of lifecycle with both favorable and unfavorable seasons. A comparison between a conventional photothermogram and a newly formulated photohydrogram or photohygrogram demonstrates that even though sufficient heat is available, scarcity of water and thus scarcity of foliage should force the cricket to maintain diapause at intermediate temperature. The results suggested that high t_0, large body size, and multi-ennial lifecycle mutually affect each other and formulate a unique adaptation under such an extreme environment.

Keywords Developmental threshold · Diapause · Life history · Tropical highland · Voltinism

Introduction

Tropical insects are often believed to have a continuous lifecycle without diapause because tropical areas are hot and wet compared with temperate or subarctic areas. However, diapause or even photoperiodism appears fairly common in tropical insects as an adaptation to fluctuating habitat conditions despite their small amplitudes (Denlinger 1986). Diapause is programmed developmental arrest prepared in advance to tolerate stress conditions before the stressful conditions actually arrive, unlike coma or quiescence (Danks 1987). Insects frequently rely on photoperiodism over temperature fluctuation as environmental token stimuli because

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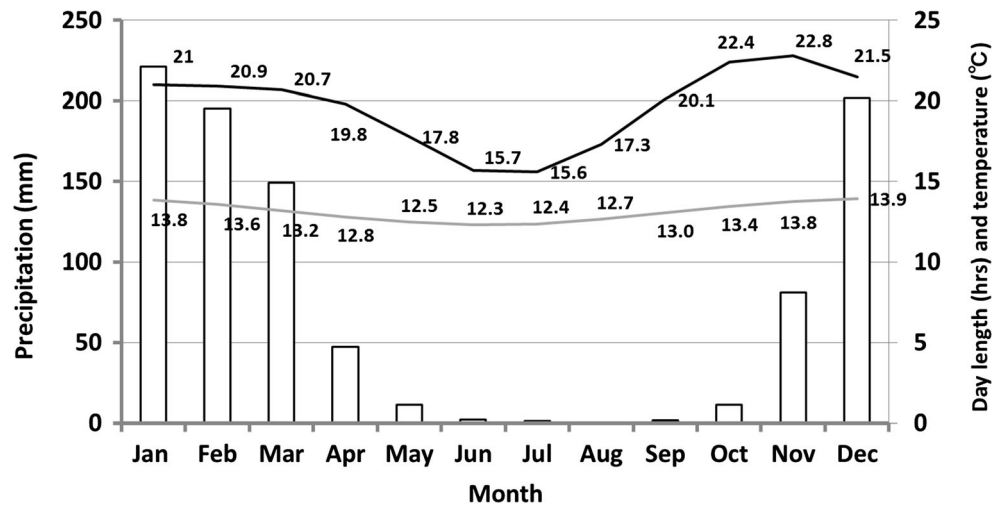
photoperiod provides more stable seasonal cues than temperature. Once insects enter diapause, they feed little and their metabolism becomes suppressed. Because insects are small and have a short life, it is important to enter diapause well before the unfavorable season actually arrives.

Crickets have been used as a favorable model to study seasonal adaptations (Masaki 1961; Tanaka 1983). Various overwintering stages are known in crickets in temperate zones, for example, egg-overwintering in *Teleogryllus emma*, nymph-overwintering in *T. occipitalis*, and overwintering at any developmental stages in *Gryllodes sigillatus*, which lives in hot spring areas in template areas (Murai and Ito 2011).

In addition, voltinism, which is the number of generation per year, is another important factor to determine the life history of insects. If certain species requires several years to complete their life span, they may undergo several diapause stages in their lifecycle. Many studies report voltinism in crickets, some bivoltine, other univoltine, and still others semivoltine (Masaki 1972; Masaki and Ohmachi 1967; Okamoto et al., 1956), but most studies have employed North American, Australian, or Japanese species that inhabit temperate areas with considerable annual precipitation.

Climate in tropical highlands fluctuates with small amplitudes in temperature throughout the year. We consider Chitedze, Malawi, as a model habitat that is characterized by

cool summers (22.8 °C), mild winters (15.6 °C), and an elevation of 1149 m a.s.l. Between June and September, however, the precipitation is practically nil and the soil becomes powdery. Namely, in addition to a cool climate, the land is characterized by an intense dry season from May to October (Fig. 1). No crops or green vegetation except some trees remain in this period and most insects are subterranean (Takeda unpublished observation).

Annual difference in photoperiod is 1.6 h (Fig. 1). On the other hand, the level of precipitation fluctuates with a robust seasonal pattern. As Tanaka et al. (1987) have demonstrated in *Stenotarsus* beetles subtle differences in photoperiod are still effective for the timely initiation and termination of diapause in a tropical environment in Panama.

Another critical point of difference is that the annual cycles of photoperiod and precipitation in Chitedze the cricket *G. argenteus* experiences have a time lag (i.e., acrophase in the dry season in Chitedze lies between May and October, but that of short day length less than 13 h lies between April and September). Therefore, the same critical photoperiod for diapause initiation and termination may be fatal. The wet–dry transition occurs in April when the photoperiod including 1 h of twilight is 12.8 h, whereas the dry–wet transition occurs in November when the photoperiod is 13.8 h. If the insect adopts the critical photoperiod as 12.8 h, the same

Table 1 Developmental parameters calculated in two ways: (1) a conventional method based on the assumption that poikilothermic development depends on a fixed heat mass (K) above developmental zero (t_0) and (2) Ikemoto and Takai (2000)'s new method

	Conventional method	Ikemoto and Takai (2000)
t_0	19.38	20.19
K	625	616.03
Equation derived from line fitting	$y = -0.031 + 0.0016x$[a]	$y = 20.189 + 616.03x$[b]
Voltinism allowed	0.6816	0.3977
Years required for a generation	>1.467	>2.516

[a] y-axis: developmental quotient = $1/D$ where D is the period of development in days. x-axis: ambient temperature in °C

[b] y-axis is DT, and x-axis is D

photoperiod recurs in August, which is in the midst of dry season. The population will be wiped out in September when the drought continues. Therefore, a simple photoperiodic solution would jeopardize survival. To find an alternative adaptation for species in such a harsh habitat, we investigated the critical factors that support the life history of this species.

Materials and methods

Insects

The African silver cricket, *Gryllus argenteus*, collected in Chitedze, Malawi (33° 33′ E, 13° 59′ S, and 1149 m a.s.l.) were reared in the laboratory on artificial diets (RC4 and MF, Oriental Yeast Co.). They were kept with sufficient food and water in transparent plastic containers (12.5 cm in diameter and 6 cm in height). The containers were placed in photoperiodic cabinets controlled by an electrical timer to provide photoperiods of LD 12:12, 13:11, 14:10, and16:8 at 25 °C and LD 12:12, 14:10, and 16:8 at 30 °C.

Developmental threshold and the estimated number of generations per year

Crickets were reared at constant temperatures of 20, 23, 25, 27.5, 30, 31.5, and 34 °C under LD 16:8. Developmental quotients ($1/D$), reciprocals of nymphal period (D) at different temperatures, were fitted by a linear regression line $y = a + bx$, where y is developmental quotient and x is temperature. t_0 is the theoretical lower threshold temperature where no development would occur, i.e., $y = 0$. t_0 and required heat unit (K) were calculated after both conventional regression line fitting based on the optimal temperature range and the new method

Fig. 3 Linear regression of the development in *G. argenteus* following Ikemoto and Takai (2000)

proposed by Ikemoto and Takai (2000). Here, required heat unit is a species-specific constant and total heat mass to complete one generation.

Photoperiodic control of nymphal period

Newly hatched crickets were reared en masse at constant 25 °C either with LD = 12:12, 13:11, or 14:10. Differences in nymphal period and femur length, which symbolizes general body size under different photoperiods, were examined. Next, other newly hatched crickets were reared individually at constant 30 °C with long or short day lengths. Molting and inter-molting periods were observed to determine the developmental stage that shows developmental arrest. The dorsal abdomen of each test insect was painted with a color marker and the time of molting was recorded when the paint disappeared after molting.
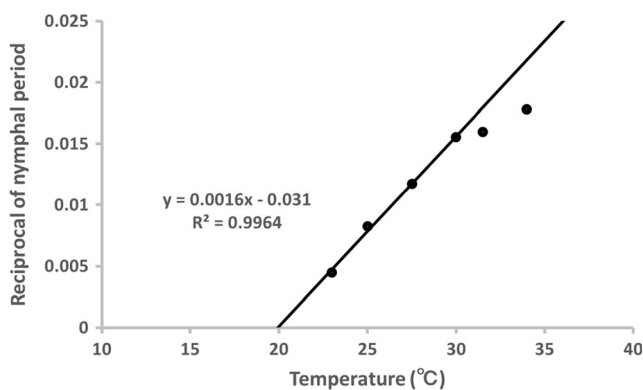

Fig. 2 The regression line of nymphal development in *G. argenteus*. The line is based on the reciprocals of the developmental period in days obtained at 23, 25, 27.5, and 30 °C. The points of 31.5 and 34 °C were excluded from calculations because these deviated from linearity
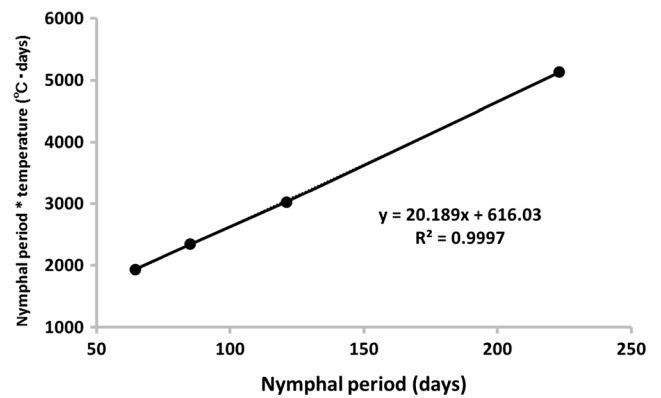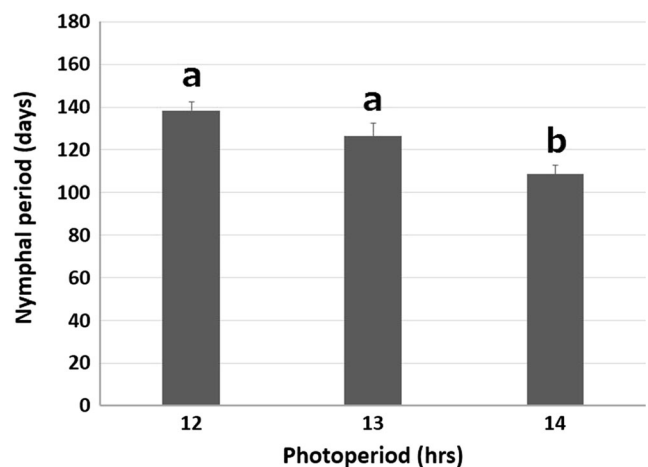

Fig. 4 Mean nymphal period of *G. argenteus* with LD 12:12, 13:11, and 14:10 at 25 °C. *Error bar* indicates standard error. *Different letters on the bars* represent different significance levels (Mann–Whitney's *U* test revised using the Benjamini–Hochberg method, $p < 0.01$). $N = 28$ (12:12), 44 (13:11), and 37 (14:10)
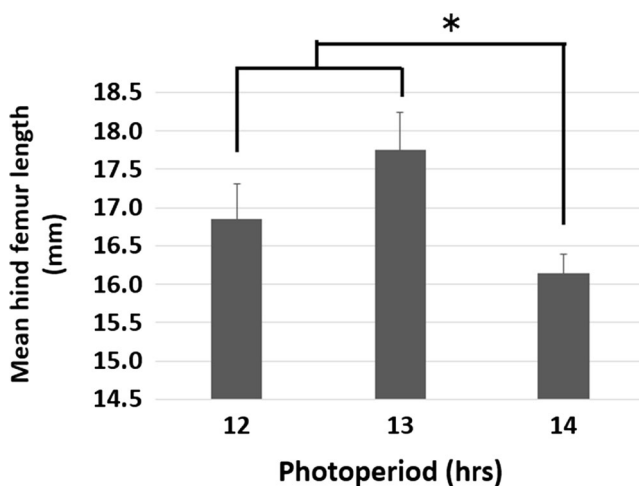
Fig. 5 Mean femur length of adult *G. argenteus* reared with LD 12:12, 13:11, and 14:10 at 25 °C. *Error bar* indicates standard error. *Asterisk* indicates that two groups are significantly different (*t* test revised using the Benjamini–Hochberg method, *p* < 0.01). *N* = 28 (12:12 and 14:10) and 33 (16:8)

Results

### Developmental threshold and the estimated number of generations per year

Developmental parameters were calculated using two methods (1) a conventional regression analysis based on the assumption of poikilothermic dependency on fixed heat mass (*K*) available above developmental zero temperature (t_0) and (2) a new method proposed recently by Ikemoto and Takai (2000). The first method is based on the above mentioned poikilothermic law, but it tends to misfit at temperature ranges above the physiological optimum or sometimes below the optimal range. These ranges were excluded from the calculation. The latter method attempted to accommodate a wider range of temperature to better fit the natural change in temperature. In the present case, however, the natural range of temperature fluctuation was small and it never runs out of the physiological range, both methods should suggest good fits (Table 1). The crickets reared at 20 °C failed to complete the nymphal stage, just like *Gryllus bimaculatus* shown by Behrens et al. (1983). Furthermore, we observed that many other cricket species failed to complete their nymphal stage at 20 °C (data not shown). Nymphal periods (*D*) at constant 31.5 and 34 °C were eliminated from the calculation for linear regression ($y = a + bx$, where $y = 1/D$, and x = ambient temperature) because of the developmental delay indicated by suppression of development at this range (Fig. 2), although the natural range never steps into this range. Based on the developmental rates at four constant temperatures, the lower threshold temperature for development ($t_0 = -a/b$) and required heat units ($K = 1/b$) were first calculated using a conventional method (Fig. 3). Lower developmental threshold (t_0) was calculated as 19.38 °C and 625 degree-days, but these were 20.19 °C and 616.03 degree-days based on Ikemoto and Takai (2000). The number of generations possible at Chitedze each year was estimated as 0.398, a product of 244.98 degree-days, a cumulative temperature mass above 20.19 °C, divided by 616.03 degree-days (Fig. 1). This value means that the cricket requires 3 years to complete one generation. The conventional method gave an estimate of 0.682 generation and 2 years are required at minimum.

Photoperiodic control of the rate of nymphal development

Nymphal stage of the crickets reared at LD 12:12 and 13:11 was significantly longer than that at LD 14:10 at constant 25 °C, and nymphs sometimes persist more than 6 months

Fig. 6 Duration of each instar in *G. argenteus* with LD 12:12 (*light columns*) and 16:8 (*dark columns*) at 30 °C. *Asterisks* indicate significant differences in the duration of instars between LD 12:12 and 16:8. (Mann–Whitney's *U* test revised using the Benjamini–Hochberg method *p* < 0.05) and *error bars* indicate standard error. The number of samples is shown under each bar
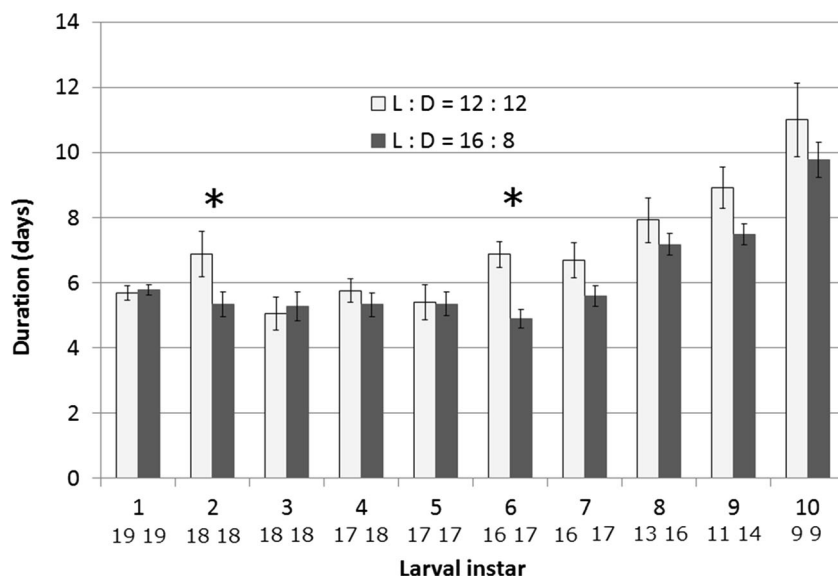
(Fig. 4). The critical day length that controlled nymphal development was estimated between 13 and 14 h. In addition, the hind femur length of adult crickets reared at LD 12:12 plus 13:11 was significantly longer than that at LD 14:10 at constant 25 °C (Fig. 5). The durations of the second and sixth instars at LD 12:12 were significantly longer than those at LD 16:8 at 30 °C (Fig. 6). Duration of the ninth instar at LD 12:12 was longer than that at LD 16:8 ($p = 0.082$). The number of instars ranged between 9 and 11 at LD 16:8 ($N = 13$) and between 9 and 13 at LD 12:12 ($N = 7$) (Fig. 7).

Discussion

Developmental parameters of *G. argenteus*

It is common to see that sublethal high temperatures exert developmental suppression, and this was also the case with *G. argenteus* where 31.5 and 34 °C induced submaximal rates of development (Fig. 2). Based on the four lower temperatures than these, t_0 was calculated as 19.38 °C using a conventional regression analysis. The new method proposed by Ikemoto and Takai gave a result of 20.19 °C (Table 1). This fell in an extremely high range among those investigated previously (Utida 1957; Honek and Kocourek 1990). Only stored product insects (Kiritani 1997) and Mediterranean insects (Bodenheimer 1927; Behrens et al. 1983) had high values in a similar range: *Lasioderma serricorne* and *G. bimaculatus* had t_0 values of 19.5 and 19.0 °C, respectively. Based on these parameters and meteorological data from Chitedze, a photothermograph was drawn with total 1 h of twilight added to the day length after sunrise and before sunset (Fig. 8). The critical photoperiod is estimated as LD 13:11, and this roughly separated the wet season from the dry season, but the problematic period was September–October, when the photoperiod exceeds 13 h but there is still no precipitation. Additional
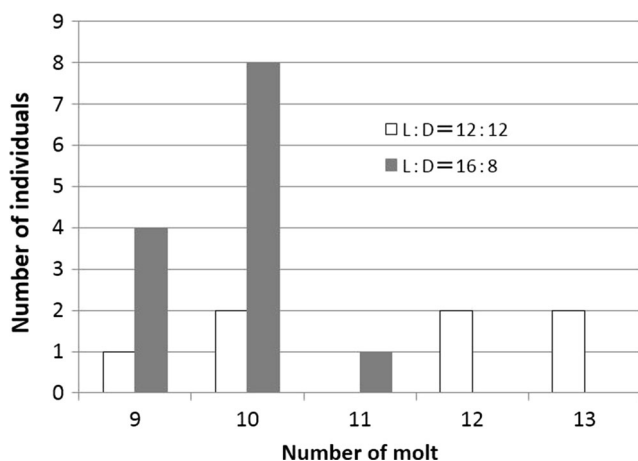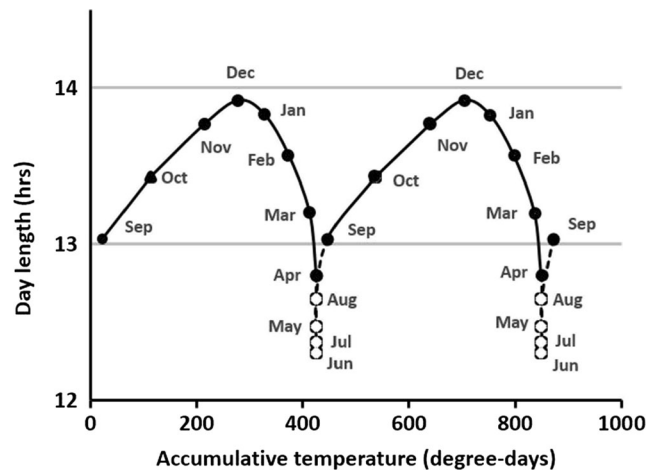

Fig. 8 A photothermographic illustration of Chitedze, Malawi. Between May and October, practically no precipitation is accumulated. Months indicated by *open circles* and *broken line* are those where temperature falls below t_0, and, therefore, no development occurs

adaptation is required to prevent resuming development at this time.

Photoperiodic control of nymphal development

Day length during the dry season in Chitedze was about 12 h (Fig. 1). The prolonged nymphal period observed with a photoperiod of LD 12:12 (Fig. 4) indicated that development of this species was photoperiodically suppressed at short day length. Some larvae stayed as nymph for more than 6 months at 25 °C, LD 12:12. Furthermore, crickets reared under short day length increased their body size (Fig. 5) while adult emergence was delayed. Size increases during developmental delay have been observed in other species (Clifford 1970; Fischer 1974). Such an increase accompanied by a delay
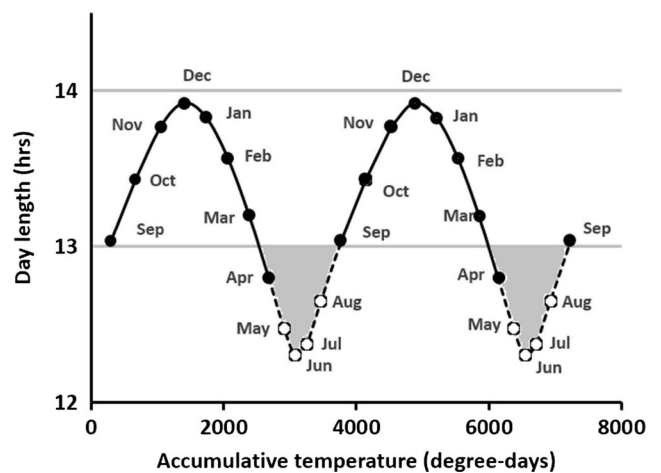

Fig. 7 Distribution of the number of molts in *G. argenteus* with LD 16:8 (*shaded*) and 12:12 (*open*) at 30 °C. $p < 0.01$

Fig. 9 A photothermograph based on $t_0 = 10.0$ as a hypothetical condition. The *gray portion* represents the season with no precipitation. The accumulation of heat units in this period could be hazardous because crickets need water to sustain their metabolism during this period with no rain, i.e., with nothing to drink and thus no food

Fig. 10 A photohydrograph, or photohygrograph, for *G. argenteus* based on accumulated precipitation and day length in Chitedze. One hour is added to the time between sunrise and sunset for effective twilight
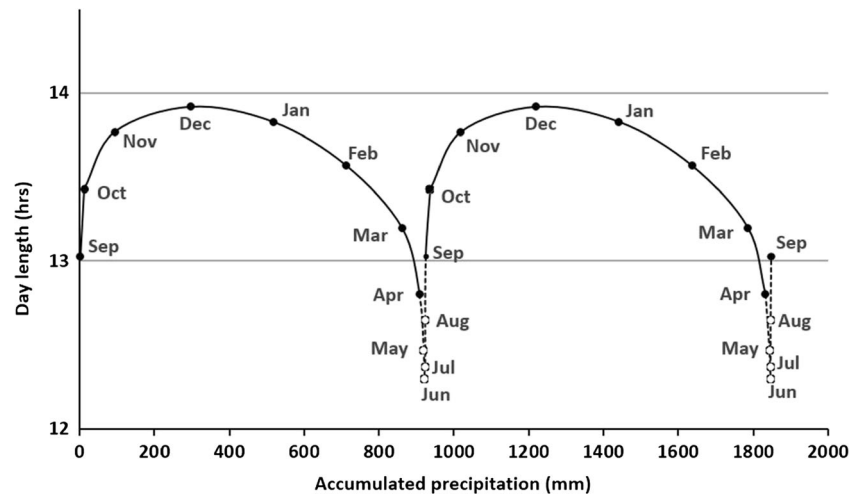
was reported by Fuzeau-Braesch (1963) in the cricket, *Gryllus campestris*, Ingram and Jenner (1976) in the damselfly *Enallagma hageni*, and by Hanna et al. (1981) in the lesser cotton leaf worm *Spodoptera exigua*.

Estimated life history

In *G. argenteus*, K is double the available heat units at Chitedze. This suggested a 2-year or possibly 3-year lifecycle. Similarly, a shortage of heat units forces the cricket *Nemobius sylvestris* to adopt a 2-year lifecycle (Alexander 1968; Brown 1978), but the temperature at Chitedze never falls below 15.6 °C. However, this season lacks precipitation, which is probably more critical to cricket's lifecycle than low temperatures. A hypothetical photothermogram was constructed (Fig. 9) based on $t_0 = 10$, which is commonplace among temperate species (e.g., Utida 1957). Comparison of the two photothermograms depicts the advantage of a high t_0 (Figs. 8 vs. 9). By maintaining an extremely high t_0, development is completely shut down during April–September. This short day phase almost coincides with the dry season. To better illustrate the advantage of high t_0, we formulated a photohydrogram or photohygrogram (Fig. 10). Figure 10 represents ecological demand by climatic factors and Figs. 8 and 9 match better than Figs. 9 and 10, because the shaded area of Fig. 9 forces development if $t_0 = 10$ °C. Three periods of developmental arrest are expected during the nymphal period. Instars 2, 6, and possibly 9–10 showed developmental retardation under LD 12:12 (Fig. 6).

The sleeping chironomid *Polypedilum vanderplanki*, which is also present in Malawi, is the only known insect to enter anhydrobiosis (Watanabe et al. 2002). Other insects in Chitedze should have other ways to escape from the dry season. We investigated the lifecycle of *G. argenteus*, because the life history of crickets in arid areas is not well studied, and crickets generally live in moist environments. Many crickets delay development in response to short photoperiod, which is

a cue for dry winters. In addition, they increase their body size by increasing the number of molts and by prolonging the nymphal period (Masaki and Walker 1987). In conclusion, in the case of *G. argenteus* photoperiodism combined with an extremely high t_0, an extremely long lifecycle, and large body size contributes to its survival in such an extreme environment. The critical stress that determines this lifecycle is not low temperature but low precipitation, as found in tropical highland such as in Malawi.

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