Occurrence of protandry in an aseasonal multivoltine moth: Implications for body-size evolution

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The behaviour of males emerging/maturing/arriving earlier than females is called protandry. Among insects, protandry has been reported only in those that are seasonal. This is probably because discrete generations, a requisite for protandry, are often reported in insects from seasonal environments. In what may be the first evidence for aseasonal multivoltine insects, we report protandry in *Opisina arenosella*, a tropical moth with discrete generations. The mean emergence time for males of *Opisina* was significantly earlier than that of females and the proportion of females in the population increased with progress in the flight period, suggesting protandry. Field-activity pattern of moths, monitored using light traps, showed that the peak activity period for males was earlier than that of females, a trend similar to that of the emergence pattern. Further, through laboratory studies on developmental biology, protandry was consistently observed over a range of temperatures (22–34°C) normally prevalent in the study area, suggesting that protandry *per se* may not be a function of seasonality. We attribute protandry in *Opisina* to sexual selection for early male emergence. We discuss why protandry among aseasonal insects cannot result from selection for body size in males or females, and suggest that protandry and smaller male body size should not be related to availability of food. The evidences provided in this communication may open opportunities for exploring reproductive asynchrony in general, and protandry in particular, among aseasonal insects.

Keywords: Discrete generations, monandry, *Opisina arenosella*, protandry, reproductive asynchrony.

AMONG monandrous insects (where females mate once in their lifetime), males should essentially mate with virgin females to be successful. Here, as early emerging males would have greater mating opportunities with virgin females, early emergence of adults from pupae may be 'selected for' in males[1–4]. Therefore, under monandry, males may tend to emerge earlier than females – a phenomenon known as protandry[2]. This sexual-selection explanation for protandry predicts the following. (1) Protandry should be associated with discrete generations, as advantages of protandry cannot be realized when mates are always available[1–3,5,6]. (2) Males should be smaller than the females[3]. The second prediction (although contested by others[7]) has been explained as follows[3]. Insects with discrete generations occur mostly in temperate regions, and, as food is available for a limited period, the life cycle may be constrained. In this case, the total development time may be the same for both sexes. Therefore, it has been speculated that if males are to emerge earlier than females due to selection for protandry, their development time should be shorter. Hence, they should have a smaller body size, if their development rates are similar. The prediction of smaller males reclines on the fact that protandry among insects is reported only in distinctly seasonal uni- or bi-voltines[8–13].

Aseasonal multivoltine insects are generally not expected to be protandrous because discrete generations are rarely reported among them[3,14–17]. In an interesting situation, we found that a tropical moth, *Opisina arenosella* (Walker) (Lepidoptera: Oecophoridae) (hereafter *Opisina*), whose larvae defoliate the coconut palm, shares all the characteristics of a protandrous insect as predicted by the sexual selection theory – monandry[18,19], discrete generations[17], and smaller male body size[20] (Figure 1); but remarkably, the insect is aseasonal and multivoltine[17], while its host plant is perennial. In this communication we report the occurrence of protandry in *Opisina* by analysing the emergence and activity pattern of moths. Thus protandry is shown in an aseasonal multivoltine insect with discrete generations. Later, some of its implications on the prediction of smaller males[3] have been discussed.

Opisina is the major leaf-feeding pest of coconut palms in India[21], Sri Lanka[22] and Burma[23]. Caterpillars feed on the leaflets and build large populations causing considerable damage to coconut palms. The species has five discrete generations in a year[17]. As larvae, males pass through seven instars and females eight, and there is no difference between sexes in each of the first seven instars with respect to duration, amount of food consumed and body weight[20]. This obviously implies similar rates of development between males and females. At 25°C, larval period for males and females is approximately 33 and 39 days respectively, and the total life cycle is completed in 65–75 days[20]. The eighth larval instar in females not only contributes to size dimorphism (Figure 1), but also delays female emergence[20]. Here, *Opisina* does not face any limitation in terms of availability of food, as coconut palms are perennial and their leaves evergreen.

In a study to record the emergence patterns of males and females along the flight period, samples were obtained from a thoroughly monitored field population infesting an orchard of about 600 20-year-old palms (K. B. Doddi Village, Channapatna Taluk; 12°37′40.0″N; 77°10′31.9″E). Samples were drawn four times during a generation of *Opisina* at an interval of nine days starting from the ap-

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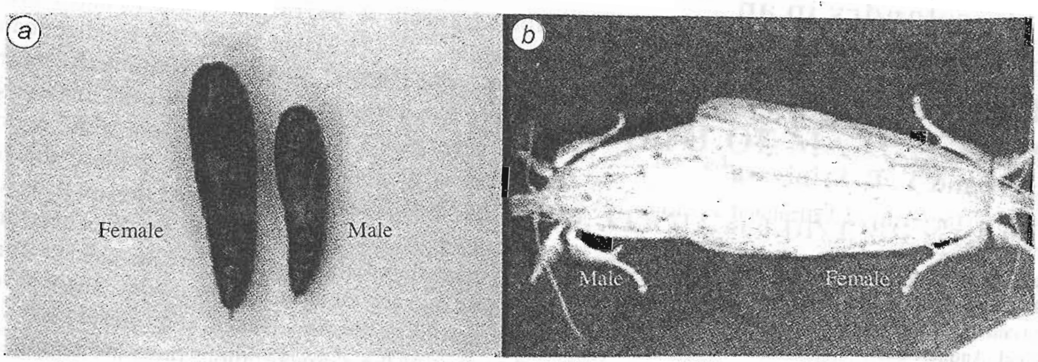

Figure 1. *a*, Photographs of male and female pupae. *b*, Mating pair of *Opisina arenosella*. Photographs show that males are smaller than females.

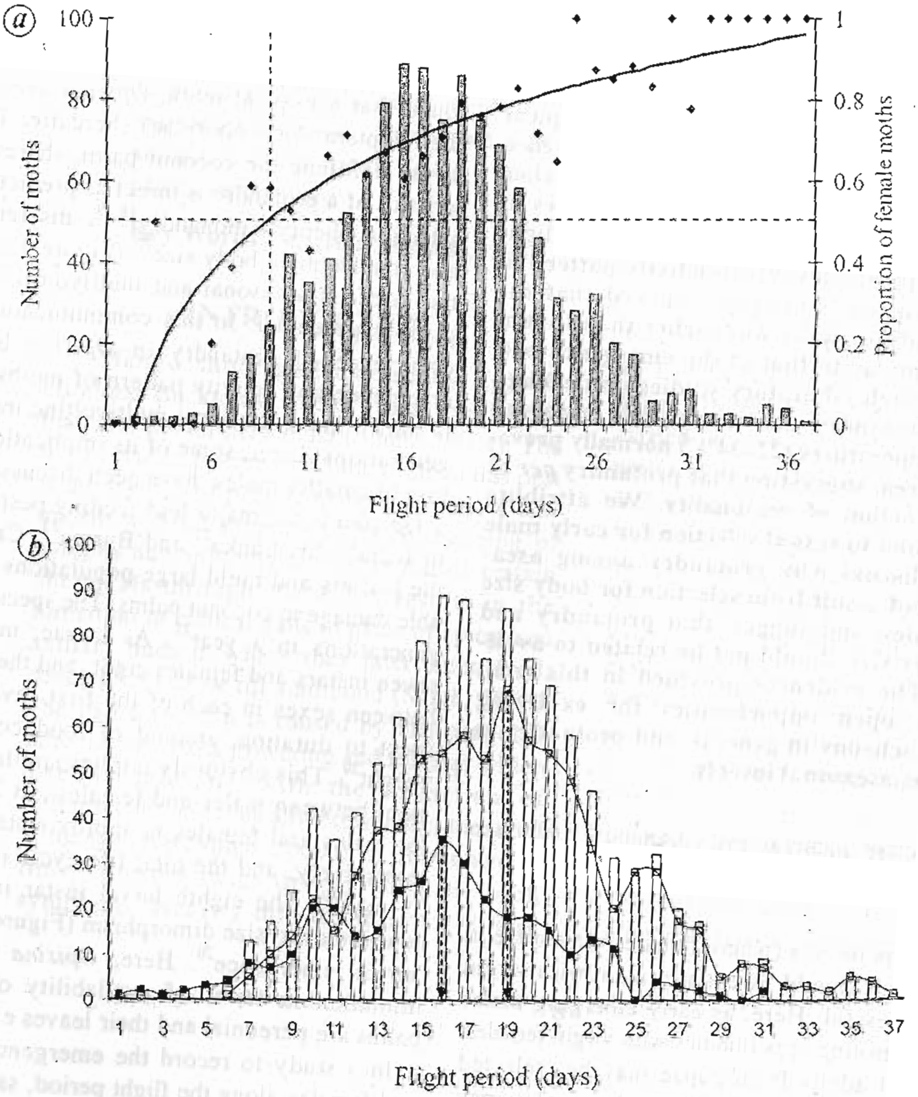

Figure 2. *a*, Overall emergence pattern of *Opisina* moths (columns) and proportion of females to total moths (dots) across the flight period. This proportion increased logarithmically along the flight period ($y = 0.3203 \ln(x)^{-0.20}$; $R^2 = 0.84$). Region below and above the horizontal dotted line indicates lower and higher proportion of females in the population respectively. Region to the left and right of the vertical dotted line indicates flight period when the proportion of females in the population is lower and higher than that of males respectively. *b*, Emergence curves of males (solid squares) and females (hollow squares) superimposed on the overall emergence (columns). Arrows indicate the respective mean emergence time of males (15.73 ± 5.25 days) and females (18.84 ± 5.60 days).

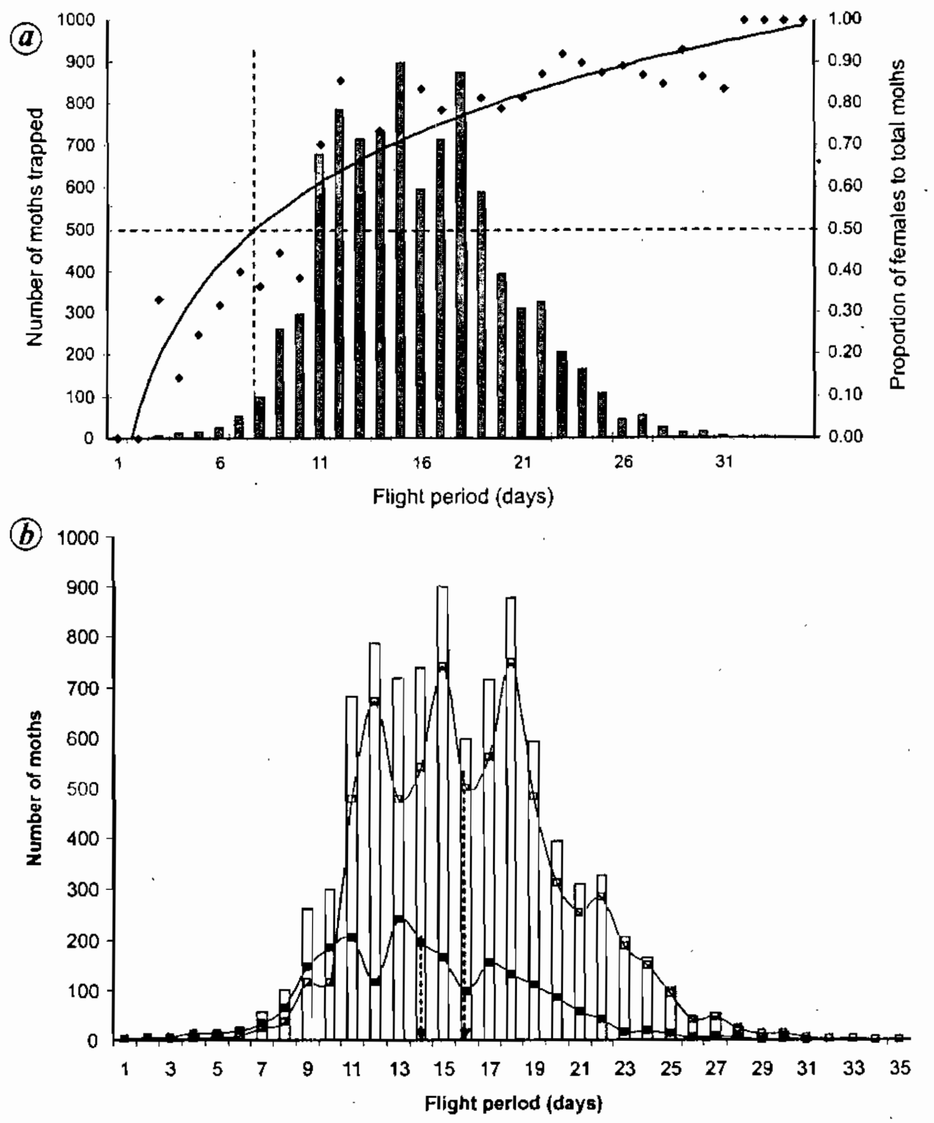

Figure 3. *a*, Overall activity pattern of *Opisina* moths (columns) and proportion of females to total moths (dots) trapped across the flight period. This proportion increased logarithmically along the flight period ($y = 0.3243 \ln(x)^{-0.17}$; $R^2 = 0.90$). See Figure 2 for further descriptions. *b*, Activity curves of males (solid squares) and females (hollow squares) superimposed on the overall activity (columns). Arrows indicate the respective mean activity time of males (14.10 ± 4.40 days) and females (16.32 ± 4.18 days).

proximate time when the first moths were to emerge (the first moth emerged on 15 April 2005). During each sampling, damaged leaflets were collected at random from different palms, bulked and brought to the laboratory to separate out the individuals. Larval and pupal stages were encountered in the first two samples; thereafter, only pupae of the current generation were found in the samples. Larvae were reared on coconut leaflets until pupation. Sexes were separated at the pupal stage and kept in containers for emergence of moths. The number of male and female moths that emerged each day was noted for the entire flight period. Data were pooled across the four samples to determine the emergence pattern.

A total of 1135 moths emerged over a period of 37 days (Figure 2 *a*). There was a single mode in the emergence of each sex (Figure 2 *b*), and the mean emergence time for males and females was 15.73 ± 5.25 and 18.84 ± 5.60 days respectively (*t*-test; $t = -8.96$; $P < 0.01$). The proportion of female moths increased logarithmically each day ($y = 0.3203 \ln(x)^{-0.20}$; $R^2 = 0.84$; Figure 2 *a*), with only females emerging during the last few days of the flight period. The sex ratio became increasingly female-biased with progress in the flight period (sex ratio (number of males per female) during the first, second and third week and for the subsequent period was 3.0, 0.67, 0.41 and 0.22 respectively). Lead/lag correlation was highest ($r = 0.93$, $n = 34$) when female emergence was advanced by three days, which also suggests a three-day difference in the mean emergence of males and females. All these evidences suggest protandry in *Opisina*.

If males emerge earlier than females, given equal adult longevity between sexes (this is seen in *Opisina*[24]), the field-activity pattern of males and females should also reflect the trend. To confirm the earlier obtained results, we conducted a field trial on the activity pattern of moths during the flight period. The study was taken up in the same orchard where samples for observing the emergence pattern of moths were drawn. *Opisina* is nocturnal and has been shown to be attracted to light irrespective of sex, mating status and age[17]. In the present study, three tray-water light traps[24], each consisting of a water-filled square tray of ~1 sq. m area and a light source in the middle, were used to trap the moths. The traps were set up 150 m apart, a few days before the emergence of the first moths. In two traps, high voltage halogen bulbs were used, while in the third trap mercury vapour lamp (200 W) was used as light source. Traps were run throughout the night from 6 pm onwards during the entire flight period – 16 April to 20 May 2005. Moths trapped during the previous night were collected every morning and stored in 70% ethanol. On two nights (4 and 5 May 2005) traps were not run throughout the night due to power failure (from 11 pm onwards). However, as it did not coincide with either the days of peak moth activity or peak activity time within a night (6–10 pm[18]), the data were used as such. Later, moths were dissected to determine the sex. Moths sampled in all the three traps were pooled for analysis.

A total of 9049 moths were trapped over a flight period of 35 days from the three light traps (Figure 3 *a*). Estimates showed the number of moths trapped to be ~1% of those active during the flight period, and hence those trapped on day n_i should have a negligible influence on the number trapped on day n_{i+j}. (The orchard under study was continuously monitored and data on population density were recorded once every 15 days. Pupal density during the generation was not less than 1 per leaflet. With ~200 leaflets per frond and ~10 infested fronds per palm, each palm contained not less than 2000 pupae. Considering that 60% pupae became moths[19], we estimated that there were ~1200 moths emerging from a single palm. As the orchard contained 600 palms, the number of moths active during the flight period in that generation should be ~720,000.) Mean trapping time across the flight period was 14.10 ± 4.40 and 16.32 ± 4.18 days for males and females respectively (Figure 3 *b*; *t*-test; $t = -20.63$; $P < 0.01$). Similar to the emergence pattern (Figure 2 *a*), the proportion of females increased with progress in the flight period ($y = 0.3243\ \ln(x)^{-0.17}$; $R^2 = 0.90$). Lead/lag correlation between males and females trapped per day was highest when the female curve was advanced by two days ($r = 0.92$, $n = 33$). These results appear similar to that of the emergence pattern, and confirm protandry in *Opisina*. However, it was necessary to verify if protandry *per se* was consistent throughout the year and not a mere artefact of the season when the study was conducted.

At any time of the year the average daily temperatures in the study area generally fall in the range 20–34°C. Therefore, we tested whether protandry was consistent at these temperatures. The developmental biology (egg to adult emergence) of *Opisina* was noted when reared at 22°C, 26°C, 30°C and 34°C. Individuals collected from the field were reared in the laboratory at the desired temperature (±1°C) and eggs of the next generation were obtained[19]. Within one day of oviposition, eggs were placed in vials (2.5 × 7.5 cm) and transferred to the BOD incubator set at a desired temperature. The vials were observed for emergence of neonates each day and the overall incubation period of eggs was noted. Neonates that emerged on the same day were transferred to coconut leaf bits in vials @ 10 per vial. After reaching third instar, the larvae were reared in jars (6 × 8 cm) and not more than 15 larvae of the same age were placed in a jar. Larvae were transferred to fresh leaflets every four days. On pupation, male and female pupae were separated and placed in containers with their respective jar numbers in the incubator. They were observed every day for moth emergence. Total time taken for development was separately worked out for the sexes. Two complete generations were reared at 22°C, 26°C and 30°C, and the parameters recorded for the second generation were used for comparisons As oviposition and survival was extremely low at 34°C, data from first generation were used. Observations were recorded from 89, 188, 296, and 84 individuals that successfully metamorphosed into adults when reared under 22°C, 26°C, 30°C and 34°C, respectively.

Results show that males completed their development earlier than that of females at each of the four temperatures tested (Figure 4). The difference in the developmental periods of males and females was 5.06, 4.12, 3.88 and 3.36 days at 22°C, 26°C, 30°C and 34°C respectively. This difference in the developmental period across sexes (*t*-test; $P < 0.05$ at all temperatures) not only proves protandry in *Opisina*, but also suggests that it occurs at all

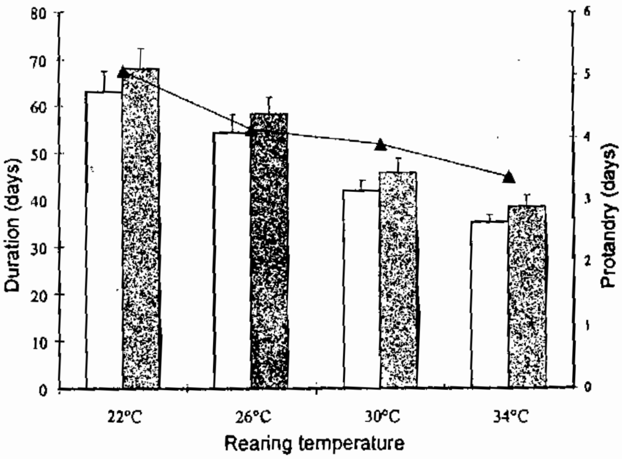

Figure 4. Total developmental period for males (unfilled columns) and females (filled columns) when reared at different temperatures (*t*-test; $P < 0.05$ for each paired comparison). Whiskers above the columns indicate 1 standard deviation. Triangles show the extent of protandry at each rearing temperature.

temperatures that are generally prevalent during a year in the study area. Thus, protandry *per se* may not be a function of temperature, the factor that has profound influence on the developmental biology of poikilotherms. Although the absolute values of protandry appear to decline with increase in temperature, the ratio of protandry to the development time of either males or females shows minor difference across the temperatures tested (for males it is 0.08, 0.08, 0.09 and 0.1; for females it is 0.07, 0.07, 0.08 and 0.09 at 22°C, 26°C, 30°C and 34°C respectively). This suggests that direct effects of temperature might not considerably influence protandry; the effects might perhaps mediate through total development time. Altogether, the study establishes protandry in *Opisina*, and simultaneously rules out the generally held belief that protandry is restricted to seasonal uni- or bi-voltines[3]. Also, it suggests that it might be redundant to link protandry and smaller male body size through seasonality or limitation for food[3].

Protandry in *Opisina* might be the outcome of sexual selection for early male emergence, as its characteristics (monandry, discrete generations and smaller male body size) agree with the general predictions of the sexual selection theory[2,3]. However, it has been proposed that protandry can also have an incidental origin[25] – it might occur when selection favours larger females (or smaller males), and the sexes have similar developmental rates. Among aseasonal insects whose life cycles are not constrained by limitation of time/food, the sexes might manipulate their developmental rates to have smaller or larger body sizes[3]; they need not compromise on reproductive synchrony. Moreover, it has been shown that protandry can risk death of virgin females[4,26]. Therefore, selection for body size might not be responsible for protandry in *Opisina*.

With seasonality out of consideration and five discrete generations per year[17], *Opisina* provides an excellent opportunity for studies on protandry.

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