Seasonal reproduction of a tropical bat, Anoura geoffroyi, in relation to photoperiod

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Summary. Anoura geoffroyi (Chiroptera, Phyllostomidae, Glossophaginae), Geoffroy’s hairy-legged long-tongued bat, were collected from September 1984 to August 1985, and these bats were found to breed seasonally in the wild on Trinidad, West Indies, at 10°N latitude. Histological examination of these samples indicated that females became pregnant in July or August, and young were born in late November or early December. The testes and epididymides were small from September to mid-April, increased threefold in weight between mid-April and late May, reached a peak weight in July, and decreased in weight in August. Spermatogenesis occurred throughout the testes of males captured from May to August. In 1990, the timing of parturition in females that gave birth in the laboratory to young conceived in the wild was similar to the timing in the field in 1984–1985. Groups of 10–13 males were subjected in the laboratory to (i) a gradually changing, civil twilight photoperiod that mimicked the natural cycle of annual change at 10°N latitude, (ii) the same gradually changing cycle of photoperiod accelerated to a six-month period, or (iii) a constant photoperiod (light 12:54 h: dark 11:06 h). These treatments began in mid-December, four months before the initiation of testicular recrudescence in the wild. In all three groups, testicular volume remained low until April, and then increased two- to threefold between late April and late June, rising to a peak in July, as occurred in the wild. Thus photoperiodic cues are not required for testicular recrudescence during the six months before peak testis size, nor is the timing of recrudescence sensitive to the accelerated pattern of photoperiodic change provided here. It is possible that other photoperiod treatments might affect reproductive timing in this species, or that other portions of the reproductive cycle are sensitive to photoperiod.

Keywords: photoperiod; tropical, seasonal breeding; bat; Phyllostomidae

Introduction

Most mammals in the temperate zone breed only seasonally, and many depend upon annual changes in daylength to enforce their seasonality (e.g. Lincoln, 1981). Many species of tropical mammals also breed seasonally, but little is known about the proximate factors that enforce seasonality in these animals (reviewed by Bronson, 1989). Annual variation in daylength decreases rapidly with latitude, until it disappears at the equator. Thus, seasonality could not be enforced by photoperiod on or near the equator. It is unknown how deeply into the tropics photoperiod can be used to regulate reproduction seasonally, or what kind of cue could do so where photoperiod is constant. Although some tropical mammals can respond reproductively to changes in photoperiod

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(Petter-Rousseaux, 1972; Van Horn, 1975; Rissman et al., 1987; Sicard et al., 1988; Wayne & Rissman, 1991), it is not known whether any use photoperiod to regulate their reproduction.

Few experimental studies have addressed these questions, and all have suffered from one or more of three deficiencies: (i) the subjects have been small, short-lived rodents or insectivores with periods of gestation short enough to permit successful opportunistic breeding without reliance on a long-term predictive cue, photoperiod or some other factor (Rissman et al., 1987; Sicard et al., 1988; Heideman & Bronson, 1990, 1992; Wayne & Rissman, 1991; Bronson & Heideman, 1992); (ii) they have tested photoperiods typical of the temperate zone rather than the tropics (Rissman et al., 1987; Sicard et al., 1988); or (iii) they have involved mammals that are only marginally tropical (Petter-Rousseaux, 1970, 1972; Van Horn, 1975), and hence experience moderately large annual changes in photoperiod.

Our objectives here are twofold. First, we will describe the annual reproductive cycle of a bat, *Anoura geoffroyi*, from the deep tropics in Trinidad at 10°N latitude. This bat has a four-month gestation period, and it must therefore initiate gonadal development, and then mate, months in advance of the phase of the annual cycle of climate, food availability and other factors that are optimal for birth and lactation. This suggests strongly that these bats rely on a predictive cue of some kind to trigger gonadal development at a particular time of the year. Second, we summarize our first test of the hypothesis that the annual cycle of testicular change of this bat depends upon changes in photoperiod. Although bats constitute almost 25% of all species of mammals, the effects of photoperiod on seasonal reproduction have been examined in only two species, both from the temperate-zone. Beasley & Zucker (1984) found that photoperiod can induce regression or development of the reproductive organ of male *Antrozous pallidus*. Racey (1978) concluded that there was no unequivocal evidence that increases in photoperiod regulated the testicular development in *Pipistrellus pipistrellus*, although he did present data suggesting that testicular development might be sensitive to particular photoperiods.

**Methods**

**Animals**

*Anoura geoffroyi*, Geoffroy's hairy-legged long-tongued bat, is in the family Phyllostomidae. The species feeds on nectar, pollen, soft fruit and soft-homed insects (Goodwin, 1944; Goodwin & Greenhall, 1961; Alvez & Gonzalez, 1970; Howell & Burch, 1974; Szirmai & Szirmai, 1978). *Anoura geoffroyi* have large eyes and presumably rely heavily on vision as well as echolocation. They are small (12–19 g), agile fliers and are capable of hovering flight. The species ranges from central Mexico south to Peru, Bolivia and east-central Brazil (Eisenberg, 1989). The population we examined came from Tamana Cave (10°28'N, 61°12'W, elevation 240 m) in central Trinidad. At Tamana Cave, *A. geoffroyi* roost in groups in chambers 15–30 m from the two large entrances. In these roosts, light intensity is low, but light from the entrances is apparent. Darlington (unpublished, 1970) reported that air temperature four feet above floor level in the chamber from which the bats in this study were collected averaged 25.9 ± 0.3°C, but air temperature at the ceiling may be several degrees higher. Darlington (unpublished 1970) also described bat activity on one night within the cave. Bats of most species (apparently including *A. geoffroyi*) were flying within the cave from about 1 h before sunset. Emergence from the cave began at about sunset, and all *A. geoffroyi* had left within 40 min of sunset. Bats returned from midnight to about dawn. The population of *A. geoffroyi* in the cave has not been censused objectively, but was of the order of several thousand individuals at the time of our studies. Goodwin & Greenhall (1961) listed nine additional species found in the cave.

Rainfall on central Trinidad is seasonal, with a dry season typically from January to April or May, and a wet season from June to December (Fig. 1). Monthly rainfall during field collection in 1984 and 1985 was typical (Fig. 1). Mean monthly temperatures range from 24.6 to 26.6°C (Piarco Meteorological Service, Trinidad).

**Field data collection**

Samples of bats were collected from the walls of the cave with a hoop net monthly from September 1984 to August 1985 (Table 1). Individuals that were obviously subadult in appearance or body weight were released. In each month, the sex ratio of bats in one sample with the net was recorded. Bats were brought back to the laboratory in darkened wire mesh cages, and then held in larger (80 cm x 80 cm x 80 cm), darkened flight cages and provided with fresh fruit and water until they were killed and autopsied. Body weights were recorded for all individuals. Reproductive organs (paired testes with attached epididymides and uterus with attached ovaries) were removed, fixed for 18 h in Bouin's solution (Humason, 1972), washed twice in 50% ethanol, stored in 70% ethanol and weighed later. Two male and two female reproductive tracts from each month were dehydrated in a graded alcohol series, embedded in paraffin, serially sectioned at 5–8 μm and stained with haematoxylin and eosin, periodic-acid Schiff's Reagent or Masson's trichrome (Humason, 1972). In males, at least 60 sections evenly distributed throughout the testes were examined.

**Table 1. Sample sizes, collection dates and reproductive status of *Anoura geoffroyi* captured in 1984 and 1985 at Tamana Cave, Trinidad**

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of males</th>
<th>Number of females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Sep</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>17 Oct</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>14 Nov</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>12 Dec</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>1985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Jan</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>13 Feb</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>20 Mar</td>
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<td>0</td>
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</tr>
<tr>
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<tr>
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<td>0</td>
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</tr>
<tr>
<td>28 Aug</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

**Long-term husbandry**

In October 1989, a sample of bats was collected at Tamana caves and transported to the University of Texas at Austin. Bats were housed in groups of 15 or less in light-sealed flight cages ventilated by an attached, exterior fan which pulled air in past a series of baffles, through the cage and out past another series of baffles. Each cage had a feeding and flight area 87 cm long, 62 cm wide and 55 cm high which was fit during part of each day. It was connected at one end by an opening 23 cm wide by 14 cm high to a roosting area 30 cm long, 62 cm wide and 55 cm high which received light only through the opening to the flight area. The walls and ceiling of each cage were lined with flexible
Results

Deciduous trees were the only species of plants grown in the area of this study. These trees were planted in September and October to increase their total height and avoid exposure to the elements. The trees were watered with a uniform distribution of water from an overhead irrigation system. A timer was used to record the amount of time each tree was watered.

The average height of the trees was measured weekly from September to December. The data was recorded in a table and analyzed using statistical methods. The results showed a significant increase in tree height over the study period.

Reference:

Laboratory data

In all three groups of bats, the pattern of testicular recrudescence and regression mimicked the pattern observed in the wild (Figs 2 and 3). There was a 20% increase in testis volume between January and April, and a repeated measures analysis of variance indicated that this trend was statistically significant ($F = 7.77; P < 0.0001$). For the full data set (January to July; later months were not included because of the change in the experimental design at the end of July), the main effect of time in a repeated measures analysis of variance was highly significant ($F = 58.27; P < 0.0001$). The main effect of photoperiod treatment was insignificant ($F = 0.01; P = 0.99$), as was the interaction between the two main effects ($F = 1.378; P = 0.09$). Thus, neither constant photoperiod from the time of the winter solstice nor acceleration of the normal photoperiod cycle to six months affected the timing of testicular recrudescence.

As stated earlier, this experiment was redesigned in late July to initiate a longer term, 3-year study. These changes (Fig. 3) are relevant here only to note that they occurred after maximum testis size had been achieved.

Discussion

Reproduction in Anoura geoffroyi on Trinidad is clearly seasonal in the wild. Males undergo a pronounced cycle of spermatogenesis and change in testis and epididymis weight, with a peak in July. Mating occurs in July and August, and females produce a single pup in late November or early December. Goodwin & Greenhall (1961) reported that all 56 females captured at Tamana Cave on 20 November 1957 were pregnant with embryos in advanced stages of development. Some of these females underwent premature deliveries and the authors judged that, if the bats had been left undisturbed, their young would have been born about one week later. Thus, the timing of births in 1957 and 1984, among wild females, and in 1990, among laboratory-housed females with embryos conceived in the wild, was very similar. This indicates that the annual cycle of reproduction of this population was similarly timed in different years.

The duration of lactation is approximately 1.5 to 2 months. However, sampling was too infrequent and the estimate of parturition dates too broad for a precise estimate.

The period of births fell within the average transition period between the wet and dry seasons, with lactation occurring during the early dry season. In seasonal wet-tropical forests, peaks in flowering tend to occur during the dry season, while peaks in fruiting and in insect abundance generally fall in the early part of the wet season (Janzen, 1976; Croat, 1975; Burkirk & Burkirk, 1976; Foster, 1985; Heideman, 1989). This suggests that the timing of reproduction in Anoura geoffroyi on Trinidad may have evolved to synchronize lactation, the period of maximum energetic demand for females, with the period of maximum abundance of nectar and pollen. Both seasonally monestrous and seasonally polyoestrous reproductive patterns have been reported in nectarivorous members of this family of bats (Fleming et al., 1972; LaVal & Fitch, 1977; Wilson, 1979; Willig, 1985a, b; Graham, 1989). The relationship between the timing of reproductive events and rainfall is variable for these species.

Data on the seasonal changes in testis size are unavailable for Anoura geoffroyi from other parts of their range. However, there are some data suggesting that females in populations elsewhere reproduce seasonally. In the Brazilian Caatingas, at latitude 7°S, pregnant Anoura geoffroyi were recorded only from September to January (Willig, 1985a). Graham (1989) reported reproductive data on females captured at scattered sites in Peru. He reported pregnant females from May to August, and lactating females from June to August and in November. Unfortunately, in both of these studies, particularly the latter, some months are represented by very few or no females. Brosset & Charles-Domique (1990) noted that females in a colony of Anoura geoffroyi had 'large young' in November at 4°N latitude in French Guiana. Several other authors, reporting on data from only one or two months at sites scattered over the geographic range of these bats, have found pregnant females in March, June or July, and lactating females in November or December (reviewed by Wilson, 1979). In general, these data suggest that seasonal breeding may be common in this species, with geographic variation in timing.

The timing of testicular recrudescence in the laboratory mimicked that in the wild, without being influenced by our experimental manipulations. We conclude, first, that the annual reproductive cycle of male Anoura geoffroyi does not require changes in photoperiod during the six months before the peak in testis size. Second, the fact that onset of testicular recrudescence could not be advanced by accelerating the rate of change in daylength suggests that the annual reproductive cycle of male Anoura geoffroyi may be insensitive to changes in photoperiod. Our results do not eliminate the possibility that photoperiod regulates the male reproductive cycle in this species. First, the reproductive cycle of these bats may resist compression into a six-month cycle, but this seems doubtful (e.g. Gwinner, 1986). Second, it is possible that our treatments did not include photoperiods both above and below a critical daylength for this species, or were of insufficient duration. We lack precise information both on the levels of light individual bats experience in the wild and on their threshold of sensitivity to light intensity. Third, photoperiod may entrain an endogenous circannual rhythm of reproduction during some other phase of the annual cycle (e.g. Malpueux et al., 1989), or these bats may have a seasonal period of refractoriness to photoperiod unrelated to circannual rhythms, as occurs in golden hamsters (e.g., Reiter, 1980; Elliott & Goldman, 1981; Stetson & Watson-Whitmyer, 1981; Steger et al., 1985).

In summary, Anoura geoffroyi on Trinidad has a pronounced seasonal cycle of reproduction, with births occurring at approximately the same time in each year in the wild. This cycle must be either directly driven by some environmental cue or cues other than photoperiod, or it must use an endogenous rhythm that is entrained by some environmental cue, which might or might not be photoperiod. Further work is necessary to separate these possibilities.
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