Diel flight periodicity and the evolution of auditory defences in the Macrolepidoptera

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We measured the 24-h flight activity patterns of 84 species of Nearctic Lepidoptera representing 12 ultrasound-earless and seven ultrasound-eared families to examine the evolution of the diel flight periodicities (DFPs) and auditory defences of these insects. Most species tested showed mixed DFPs (flight during day and night hours) with few being exclusively nocturnal. With the exception of one geometrid moth and one arctiid moth, only the butterflies (Papilionoidea+Hesperioidea) were exclusively diurnal, supporting the hypothesis that this trait has resulted from their lack of ultrasound-sensitive ears, structures that in most other Macrolepidoptera provide auditory defence against aerially foraging, insectivorous bats. Eared species showed the highest tendency to fly during both day and night, a trait we suggest results from their possession of both visual and auditory defences. Earless moths were almost exclusively nocturnal and may be constrained to the night because of their size and tendency for ground-hugging flight, which may provide protection against bats, but would be liabilities against diurnal, visually hunting predators. Ultrasound sensitivity not only appears to protect eared moths from aerial predators (bats) but also protects them from terrestrial predators by allowing the moths to remain in the air during the night, and, we suggest, is responsible for the success of this group of Macrolepidoptera.

Diel periodicity, the patterning of 24-h activity, is a fundamental characteristic of insect biology that has attracted research at both proximate (e.g. physiological) and ultimate (e.g. evolutionary) levels (for reviews see Dreisig 1986; McNeil 1991). While 'activity' includes relatively inconspicuous behaviours such as feeding or pheromone release, it is the act of moving (e.g. flight) that presumably most exposes animals to their would-be predators (Sakaluk & Belwood 1984; Fleishman 1986; Acharya & McNeil 1998). Because the day and the night present considerably different types of potential predators, the sensory defences of flying insects should be influenced by their (and their predators') diel flight periodicity (DFP). For primary defences (i.e. those that operate before a predator is aware of a potential prey; Edmunds 1974), diurnally flighted insects should rely more upon vision to detect the approach of potential predators, whereas nocturnal taxa should favour auditory or chemical systems (Svensson 1996). To examine the evolution of diel sensory adaptations in insects it is necessary to know their entire 24-h flight patterns, but such observations are surprisingly few. Most periodicity

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studies focus upon the activities of species (usually economically important ones) during only the day (photophase) or night (scotophase) portions of their diel cycle, with the result that many taxa have been labelled 'diurnal' or 'nocturnal' without knowledge of their complete 24-h activity patterns (Ehnbom 1948; Powell 1973; Dreisig 1986; Chinery 1989; Nielsen & Common 1991; Aiello 1992; Svensson et al. 1999). If these labels are used to imply specific diel periodicities (e.g. 'diurnal' means no nocturnal activity), this may lead to assumptions about their sensory adaptations (Ehnbom 1948; Powell 1973; Horridge et al. 1977; Cutler et al. 1995; Kreusel & Häuser 1997; Schmitz & Bleckmann 1998; Fullard & Dawson 1999).

Lepidoptera can serve as useful models for these questions because they show a diversity of both flight habits and sensory adaptations (Svensson et al. 1999). As most Lepidoptera have short adult life spans, selection should favour traits that enable individuals to maximize their time flying in search of mates and oviposition sites. Sensory defences against diurnal and nocturnal predators should be important factors in maximizing this flight time. A profound sensory difference within the Lepidoptera is the presence of ultrasound-sensitive ears in most moths compared to their absence in most butterflies

(Scoble 1992) (the purported ears of certain nymphalid butterflies (Otero 1990) are not ultrasound sensitive (Swihart 1967; Ribarič & Gogala 1996) and appear to be used in a social context (Monge-Nájera et al. 1998; Yack et al. 2000)). Ultrasound-sensitive ears alert their owners (usually moths, but see Yack & Fullard 2000) to the presence of echolocating, insectivorous bats (Roeder 1967) and their absence in most butterflies (Rhopalocera) has been argued to have selected for these insects' presumed diurnal preferences (Fullard 1988, 1998; Morrill & Fullard 1992; Fullard et al. 2000; Yack & Fullard 2000). The basic assumption of this hypothesis however is untested because the DFPs of most butterflies are unknown and the nocturnal activity of certain hedyloid butterflies (Aiello 1992) suggests that night-flight may be more common than believed in the Rhopalocera.

In this paper, we use a modified technique (Fullard & Dawson 1999) of near infrared video recording (Conner & Masters 1978; Webster & Conner 1986; Riley et al. 1990, 1992) to monitor the 24-h flight activity patterns of 84 species of Nearctic Lepidoptera. We use the resultant flight actograms to test the assumption of diurnality in butterflies and to test whether eared Lepidoptera differ in their diel flight periodicities from those of earless species. We also map our flight characters onto recently published phylogenies of the Lepidoptera (Kristensen & Skalski 1998; Weller et al. 1994) to examine the evolution of DFP and auditory defences in the Macrolepidoptera.

METHODS

Animals

We conducted this study during the summer months (June–September) from 1997 to 2000 at the Queen's University Biological Station (QUBS) in southeastern Ontario, Canada (44°34′N, 79°15′W). We collected Lepidoptera from wild populations using hand nets during the day or from ultraviolet lights erected on the QUBS property during the night. We identified species using Forbes (1960), Covell (1984), Ward et al. (1974), Riotte (1992) and Opler (1998) and compared them to vouchers in the insect collections at QUBS. We categorized species as eared if they possessed an externally visual tympanic membrane, or belonged to families with species that have been demonstrated to show an auditory sense (for reviews see Eggers 1919; Roeder 1974) because exceptions to these ear classifications are rare and exist only in taxa with extremely unusual life histories (e.g. bat-free habitats: Clarke 1971; Fullard 1994; Surlykke et al. 1998).

Air temperatures are continuously recorded at QUBS as part of the meteorological monitoring function of the station. We obtained the air temperatures at 20-min intervals from 1 June to 31 Aug 1998 from the station files and used these to compute the average diel temperatures throughout the summer.

Measurements of Diel Flight Periodicity (DFP)

Within 18 h of capture, we placed specimens into chambers made of fibreglass screening shaped into half cylinders measuring 15.2 cm tall with a 6.5 cm radius and backed by white Styrofoam®. We inserted a microcentrifuge tube filled with a dilute sucrose solution through the back of the chamber and allowed the insects to feed ad libitum for the 24-h observation period. Cages were visually separated from each other by sheets of cardboard. We placed the chambers in a $220 \times 270 \times 200$ cm plastic screen tent in a partially open forest that was isolated from human activity and exposed to ambient environmental conditions. Temperatures recorded inside and outside the tent three times every day of testing never differed by more than 2° throughout the summer months.

We positioned an automatic light-sensing video camera (Sanyo VDC-9212) 60 cm in front of the chambers and focused it on the moths within the chambers (Fullard & Dawson 1999). The camera uses ambient light during the day and automatically converts to a built-in set of near infrared lights during the night ($\lambda=980$ nm, wavelengths not received by lepidopteran eyes: Horridge et al. 1977; Scherer & Kolb 1987; Arikawa et al. 1999). We allowed the animals to be tested to acclimatize in their cages for 30 min (without observers present) before we began videotaping and at no time throughout the 24 h were the moths disturbed. Whenever possible, no two individuals of the same species were used on the same day and to avoid possible pheromonal interactions, we never tested different conspecific genders on the same day. The output of the camera was fed into a video-cassette recorder at another location and the 24-h activities were recorded using 10-h tapes (BASF T-200). After the insects had been observed for one 24-h period, they were cold-anaesthetized, killed and preserved by injection with 70% ethyl alcohol (only the data from animals that were not dead or moribund following the observation period were used).

For analysis, we played the tapes back at high speed and recorded flight as the number of minutes per 10-min period (bin) that an individual showed at least one movement of at least one body length accompanied by wing beats during the minute (e.g. a score of 50% indicates an animal that showed at least one flight movement during 5 of the 10 min but not necessarily for the entire 5 min of that period). We express DFP as the degree of nocturnality, that is, the percentage of an individual's 24-h flight activity that occurred from the end of twilight in the evening to 40 min past sunrise in the morning (individual twilight times for the testing day of each specimen were provided by the Herzberg Institute of Astrophysics, National Research Council of Canada <http://www.hia.nrc.ca>). We explain our reasons for choosing these times below (see Results). Diurnal movements were occasionally displayed by some specimens (which had no means of concealing themselves during early morning hours; cf. Quiring 1994), therefore we defined DFP using the following categories: (1) exclusively diurnal (0–10% nocturnal); (2) mixed, primarily diurnal (10–50% nocturnal); (3) mixed, primarily nocturnal (50–90% nocturnal) and (4) exclusively nocturnal (90–100% nocturnal). We also computed the diel diversity, a value that describes the flight tendency of

individuals throughout the entire 24-h cycle. We computed this value as the ratio of the insect's percentage of nocturnality to its diurnality (the larger of the two values being the denominator). Theoretical values range from zero (exclusively nocturnal or diurnal) to one (equally nocturnal and diurnal).

We feel that the use of cages is a valid method for monitoring the timing of flight patterns for the following reasons. First, the videotapes revealed few signs of startle-induced flight during the 24-h observation period, and following the sessions, over 95% of the individuals tested were in good condition. We accounted for occasional movements of specimens (perhaps in search of preferable roost locations) by allowing up to 10% of daily flight activity before defining the DFP as exclusively diurnal or nocturnal (see above). Second, the use of cages for behavioural monitoring of insects is an established technique (e.g. mating studies: Webster & Conner 1986; Conner 1987; Svensson 1996; flight studies: Edwards 1962). Riley et al. (1992) compared the nocturnal activities of an Indian noctuid, *Helicoverpa armigera*, and recorded similar results between field collections of wild individuals and those held in flight cages. Similarly, our flight actograms resemble the flight patterns of conspecifics in free-flight studies (Cardé et al. 1974; Morrill & Fullard 1992). Third, as stated earlier, because the focus of our study was the timing of flight activity rather than the total amount of flight activity, we feel that cages did not influence this characteristic.

Bat Activity

We monitored the emergence of approximately 1200 wild little brown bats (Vespertilionidae: *Myotis lucifugus*) from their maternity colony in an abandoned farmhouse near our study site (Barclay 1982). We chose this bat because it is a common species, easily observed and has been documented feeding on moths in this area (Belwood & Fenton 1976). Observers were positioned beneath each of two roof exits with a hand counter and recorded the number of bats as they exited every 5 min. These observations were repeated on 8 evenings in 1997 and 2 evenings in 2000.

Phylogeny

We mapped our values of nocturnality onto the lepidopteran phylogenies proposed by Kristensen & Skalski (1998) and Weller et al. (1994) using MacClade 3.1 (Maddison & Maddison 1992) (licensed to D. T. Gwynne, University of Toronto). Only those superfamilies that we sampled with greater than one species were included. For character states, we considered nocturnality to exist in only three conditions: exclusive diurnality (0–10% nocturnal), mixed (10–90% nocturnal) and exclusive nocturnality (90–100% nocturnal). To examine this character evolution within the Macrolepidoptera we used the outgroups of the Pyraloidea (Obtectomera) and Zygaenoidea and Tortricoidea (Apoditrysia).
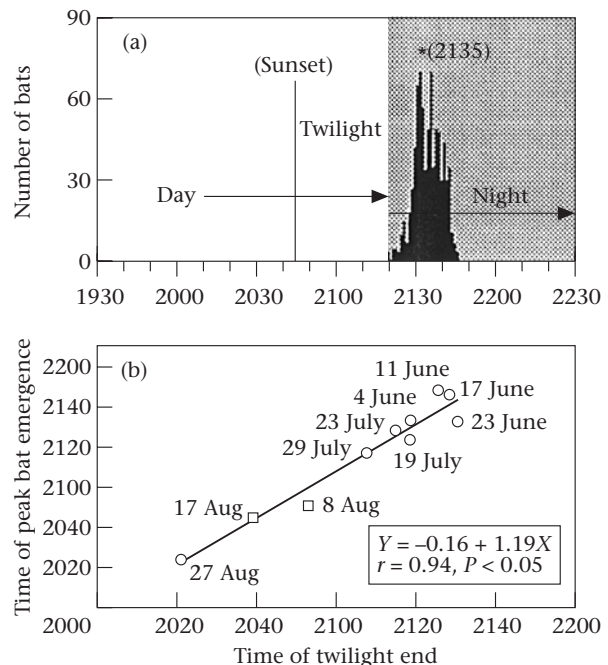

Figure 1. (a) Emergence patterns of little brown bats, *Myotis lucifugus*, on 4 June 1997 from a colony near to the study site. The time of peak emergence is indicated with an asterisk along with the definition of night as used in this paper. (b) Regression of peak emergence times of the bats from this colony and the time of twilight end for 8 nights in 1997 (○) and 2 nights in 2000 (□).

RESULTS

For this study we defined night as beginning at the end of twilight (summer range: 2030–2130 hours) because this is when a local common species of insectivorous bat (*Myotis lucifugus*) begins to fly (see Discussion). Figure 1a illustrates the emergence of individuals from a *M. lucifugus* maternity colony on one night and indicates that the colony exit begins at the end of twilight, reaching a peak emergence 15–20 min later. Figure 1b indicates that the peak emergence times of these bats are significantly dependent on the end of twilight as it changes throughout the summer as observed on 10 different nights throughout the 1997 and 2000 field seasons. For the beginning of day, we used the activity patterns of North American birds as described in Robbins (1981), whose observations indicate that the numbers of birds observed in another Nearctic locale (Maryland) reach their maxima approximately 40 min past sunrise. Using the sunrise/sunset tables for our locale, we therefore determined that day, as determined by bird activity, begins from 0600 to 0650 hours, depending on the time of summer. We computed the times of night and day for all specimens tested for the day that their particular actograms were measured.

Table 1 lists the species of Lepidoptera that were tested in this study with their auditory condition, nocturnality and diel diversity. To reduce the effects of pseudoreplication, the median values reported for families used averaged values for genera with more than one species (e.g. *Catocala*). Figure 2 illustrates the actograms of these

Table 1. Systematic ordering of the species of Lepidoptera used in this study with the number of auditory (receptor) neurons per ear, degree of nocturnality and diel diversity

	N	Ear*	% Nocturnality†	Diversity
Superfamily: Zygaenoidea			**52.1**	**0.15**
Family Limacodidae			52.1	0.15
Apoda biguttata	3	0	*54.1±46.4*	*0.30±0.45*
Tortricidia flexuosa	2	0	*50.0±70.7*	*0.00±0.00*
Superfamily: Cossoidea			**47.0**	**0.28**
Family Cossidae			47.0	0.28
Subfamily: Cossinae				
Prionoxystus robiniae	5	0	*47.0±35.4*	*0.28±0.22*
Superfamily: Tortricoidea			**75.4**	**0.18**
Family: Tortricidae			75.4	0.18
Subfamily: Tortricinae				
Choristoneura fractivittana	4	0	*84.3±12.7*	*0.21±0.20*
Sparganothis pettitana	5	0	*66.4±43.5*	*0.14±0.24*
Superfamily: Pyraloidea			**57.7**	**0.18**
Family: Pyralidae			57.7	0.18
Subfamily: Pyraustinae				
Blepharomastix ranalis	5	4	*26.1±21.2*	*0.41±0.37*
Desmia funeralis	5	4	*16.5±12.0*	*0.22±0.18*
Subfamily: Pyralinae				
Herculia olinalis	5	4	*89.3±11.3*	*0.13±0.15*
Subfamily: Crambinae				
Crambus agitatellus	5	4	*99.6±0.6*	*0.00±0.01*
Superfamily: Lasiocampoidea			**70.7**	**0.14**
Family: Lasiocampidae			70.7	0.14
Subfamily: Gastropachinae				
Phyllodesma americanum	5	0	*70.7±42.1*	*0.14±0.25*
Subfamily: Lasiocampinae				
Malacosoma americanum	5	0	*98.3±3.8*	*0.02±0.04*
Malacosoma disstria	3	0	*93.7±11.0*	*0.08±0.14*
Subfamily: Macromphalinae				
Tolype notialis	5	0	*40.9±40.3*	*0.34±0.45*
Superfamily: Bombycoidea			**90.4**	**0.01**
Family: Apatelodidae			80.6	0.01
Subfamily: Apatelodinae				
Olceclostera angelica	5	0	*80.6±43.5*	*0.01±0.01*
Family: Saturniidae			95.2	0.06
Subfamily: Citheroniinae				
Dryocampa rubicunda	5	0	*90.4±10.3*	*0.12±0.13*
Subfamily: Hemileucinae				
Automeris io	5	0	*100.0±0.0*	*0.00±0.00*
Family: Sphingidae			90.4	0.01
Subfamily: Sphinginae				
Amorpha juglandis	4	0	*99.3±1.3*	*0.01±0.01*
Ceratomia undulosa	5	0	*98.7±1.8*	*0.01±0.02*
Paonias excaecatus	5	0	*90.3±8.5*	*0.12±0.11*
Paonias myops	5	0	*73.9±32.3*	*0.17±0.13*
Smerinthus jamaicensis	5	0	*80.0±44.7*	*0.00±0.00*
Superfamily: Hesperioidea			**0.3**	**0.00**
Family: Hesperiidae			0.3	0.00
Subfamily: Pyrginae				
Epargyreus clarus	2	0	*0.0±0.0*	*0.00±0.00*
Thorybes pylades	5	0	*0.3±0.8*	*0.00±0.01*
Subfamily: Heteropterine				
Carterocephalus palaemon	5	0	*14.8±24.7*	*0.19±0.32*
Subfamily: Hesperiinae				
Hesperia sassacus	5	0	*0.4±0.6*	*0.00±0.01*
Polites mystic	2	0	*0.2±0.3*	*0.00±0.00*
Polites peckius	4	0	*0.0±0.0*	*0.00±0.00*
Thymelicus lineola	5	0	*1.0±1.0*	*0.01±0.01*
Wallengrenia egeremet	4	0	*0.0±0.0*	*0.00±0.00*
Superfamily: Papilionoidea			**1.8**	**0.02**
Family: Papilionidae			0.0	0.00
Subfamily: Papilioninae				
Papilio glaucus	4	0	*0.0±0.0*	*0.00±0.00*

Table 1. *Continued*

	N	Ear*	% Nocturnality†	Diversity
Family: Pieridae			5.3	0.06
Subfamily: Coliadinae				
Colias philodice	5	0	*5.3±7.4*	*0.06±0.09*
Subfamily: Pierinae				
Pieris rapae	5	0	*5.3±7.4*	*0.06±0.09*
Family: Lycaenidae			3.2	0.04
Subfamily: Eumaeinae				
Satyrium calanus	5	0	*0.8±1.1*	*0.01±0.01*
Subfamily: Polyommatinae				
Glaucopsyche lygdamus	3	0	*5.6±9.6*	*0.07±0.12*
Family: Nymphalidae			0.4	0.00
Subfamily: Limenitidinae				
Limenitis archippus	4	0	*0.0±0.0*	*0.00±0.00*
Limenitis arthemis	5	0	*0.3±0.7*	*0.00±0.01*
Subfamily: Melitaeinae				
Phyciodes tharos	5	0	*0.6±1.0*	*0.01±0.01*
Subfamily: Argynninae				
Speyeria cybele	5	0	*0.9±1.9*	*0.01±0.02*
Subfamily: Satyrinae				
Cercyonis pegala	5	0	*0.1±0.1*	*0.00±0.00*
Coenonympha inornata	5	0	*0.0±0.0*	*0.00±0.00*
Enodia anthedon	5	0	*1.5±3.4*	*0.02±0.04*
Megisto cymela	5	0	*0.0±0.0*	*0.00±0.00*
Satryodes eurydice	5	0	*0.7±1.5*	*0.01±0.02*
Superfamily: Drepanoidea			**89.6**	**0.14**
Family: Drepanidae			89.6	0.14
Subfamily: Drepaninae				
Drepana bilineata	4	4	*97.5±3.4*	*0.03±0.04*
Subfamily: Oretinae				
Oreta rosea	4	4	*81.7±15.1*	*0.26±0.23*
Superfamily: Geometroidea			**81.8**	**0.08**
Family: Geometridae			81.8	0.08
Subfamily: Ennominae				
Biston betularius	4	4	*91.6±11.1*	*0.10±0.14*
Ennomos magnaria	5	4	*98.2±3.3*	*0.02±0.04*
Prochoerodes transversata	5	4	*94.2±4.8*	*0.06±0.05*
Xanthotype urticaria	5	4	*71.9±23.6*	*0.31±0.21*
Subfamily: Geometrinae				
Nemoria lixaria	5	4	*50.9±25.4*	*0.46±0.23*
Subfamily: Larentiinae				
Trichodezia albovittata	5	4	*0.4±0.4*	*0.00±0.00*
Superfamily: Noctuoidea			**69.2**	**0.17**
Family: Notodontidae			95.7	0.05
Subfamily: Notodontinae				
Nadata gibbosa	5	1	*86.1±25.5*	*0.16±0.30*
Odontosia elegans	5	1	*97.9±4.8*	*0.02±0.05*
Peridea angulosa	5	1	*93.0±13.8*	*0.10±0.20*
Peridea ferruginea	5	1	*98.4±1.6*	*0.02±0.02*
Pheosia rimosa	5	1	*99.7±0.7*	*0.00±0.01*
Subfamily: Phalerinae				
Datana contracta	5	1	*95.7±7.2*	*0.05±0.09*
Subfamily: Pygaerinae				
Clostera albosigma	3	1	*95.2±8.2*	*0.06±0.10*
Family: Arctiidae			55.3	0.21
Subfamily: Arctiinae				
Cycnia tenera	5	2	*54.6±35.6*	*0.37±0.37*
Ecpantheria scribonia	5	2	*94.6±4.9*	*0.06±0.06*
Haploa confusa	5	2	*47.2±32.3*	*0.46±0.39*
Haploa contigua	5	2	*64.9±31.4*	*0.31±0.29*
Phragmatobia fuliginosa	5	2	*49.6±26.3*	*0.49±0.30*
Subfamily: Ctenuchinae				
Cisseps fulvicollis	5	2	*81.7±6.5*	*0.23±0.10*
Ctenucha virginica	5	2	*42.7±40.1*	*0.18±0.14*
Subfamily: Lithosiinae				
Hypoprepia fucosa	5	2	*87.5±12.6*	*0.17±0.19*
Hypoprepia miniata	5	2	*85.8±15.5*	*0.20±0.26*
Lycomorpha pholus	4	2	*0.0±0.0*	*0.00±0.00*

Table 1. *Continued*

	N	Ear*	% Nocturnality†	Diversity
Family: Lymantriidae			38.1	0.14
Subfamily: Lymantriinae				
Lymantria dispar	5	2	*38.1±40.9*	*0.14±0.08*
Family: Noctuidae			83.1	0.20
Subfamily: Herminiinae				
Polypogon ochreipennis	2	2	*100.0±0.0*	*0.00±0.00*
Subfamily: Acronictinae				
Acronicta americana	5	2	*82.1±13.9*	*0.25±0.21*
Subfamily: Agaristinae				
Eudryas grata	3	2	*97.7±0.3*	*0.02±0.00*
Eudryas unio	5	2	*69.9±32.4*	*0.28±0.29*
Subfamily: Amphipyrinae				
Amphipyra pyramidoides	5	2	*75.9±19.7*	*0.33±0.30*
Subfamily: Catocalinae				
Caenurgina erechtea	5	2	*67.6±28.2*	*0.29±0.17*
Catocala coccinata	2	2	*62.9±6.2*	*0.60±0.16*
Catocala cerogama	5	2	*85.0±15.6*	*0.21±0.25*
Catocala subnata	5	2	*70.9±20.2*	*0.50±0.39*
Subfamily: Plusiinae				
Anagrapha falcifera	5	2	*89.5±2.2*	*0.12±0.03*
Subfamily: Hadeninae				
Leucania inermis	5	2	*94.9±5.8*	*0.06±0.07*
Pseudaletia unipuncta	4	2	*93.4±5.5*	*0.07±0.07*
Subfamily: Noctuinae				
Feltia jaculifera	5	2	*81.0±12.4*	*0.26±0.21*
Subfamily: Ophiderinae				
Panopoda carneicosta	5	2	*82.3±18.2*	*0.27±0.34*
Subfamily: Pantheinae				
Panthea furcilla	5	2	*94.0±4.9*	*0.07±0.06*

Species values (italicized) are averages of all individuals tested (N=samples sizes), family values (roman) are medians of species means, superfamily values (bold) are medians of family values.
*Number of auditory receptors per ear (0 indicates earless state).
†Percentage of diel cycle.

species with average summer scotophase illustrated as shaded bars (nocturnality values for statistical analyses were computed from actual times on the days when actograms were done and are used in Table 1). From these data the following superfamily median DFPs were seen: (1) exclusively diurnal: Hesperioidea, Papilionoidea; (2) mixed, primarily diurnal: Cossoidea; (3) mixed, primarily nocturnal: Zygaenoidea, Tortricoidea, Pyraloidea, Lasiocampoidea, Bombycoidea, Drepanoidea, Geometroidea, Noctuoidea; (4) exclusively nocturnal: (none). DFP categories at the family level (with percentages of total number of species) were as follows: (1) exclusively diurnal (26.3%): Hesperiidae, Papilionidae, Pieridae, Lycaenidae and Nymphalidae; (2) mixed, primarily diurnal (10.5%): Cossidae, Lymantriidae; (3) mixed, primarily nocturnal (42.1%): Limacodidae, Tortricidae, Pyralidae, Lasiocampidae, Apatelodidae, Geometridae, Arctiidae and Noctuidae; (4) exclusively nocturnal (21.1%): Saturniidae, Sphingidae, Drepanidae and Notodontidae. The following species with DFPs showed exceptions to these generalizations: (1) exclusively nocturnal in DFP mixed families: *Crambus agitatellus* (Pyralidae), *Malacosoma americanum* and *M. disstria* (Lasiocampidae), *Polypogon ochreipennis*, *Eudryas grata*, *Leucania inermis*, *Pseudaletis unipuncta*, *Panthea furcilla* (Noctuidae); (2) DFP mixed in primarily diurnal families: *Carterocephalus palaemon* (Hesperiidae); (3) exclusively diurnal in primarily nocturnal families: *Trichodezia albovittata* (Geometridae), *Lycomorpha pholus* (Arctiidae).

Note the apparent paradox between species nocturnality and diel flight diversity. A species consisting of individuals that were equally exclusively nocturnal or diurnal (e.g. *Tortricidia flexuosa*) presented a mean nocturnality value of 50%, but the diel diversity of this species was zero because no individual showed a mixed DFP. Another theoretical species consisting of individuals that are all 50% nocturnal also shows a mean nocturnality of 50% but in this case, the species mean diel diversity would be one because all of the individuals in this species would have equally mixed DFPs. The two species represent extremes in diel flight patterns and the diel diversity measurement identifies their differences.

Figure 3 compares the averaged flight cycles of all exclusively nocturnal or diurnal species to the average air temperatures for every 20 min of the entire summer period that the DFPs were measured. The flight activity of exclusively nocturnal species decreased from ca. 2200 to 0600 hours corresponding to the average fall in temperatures during this period. The arrows in Fig. 3 indicate equivalent temperatures during the scotophase
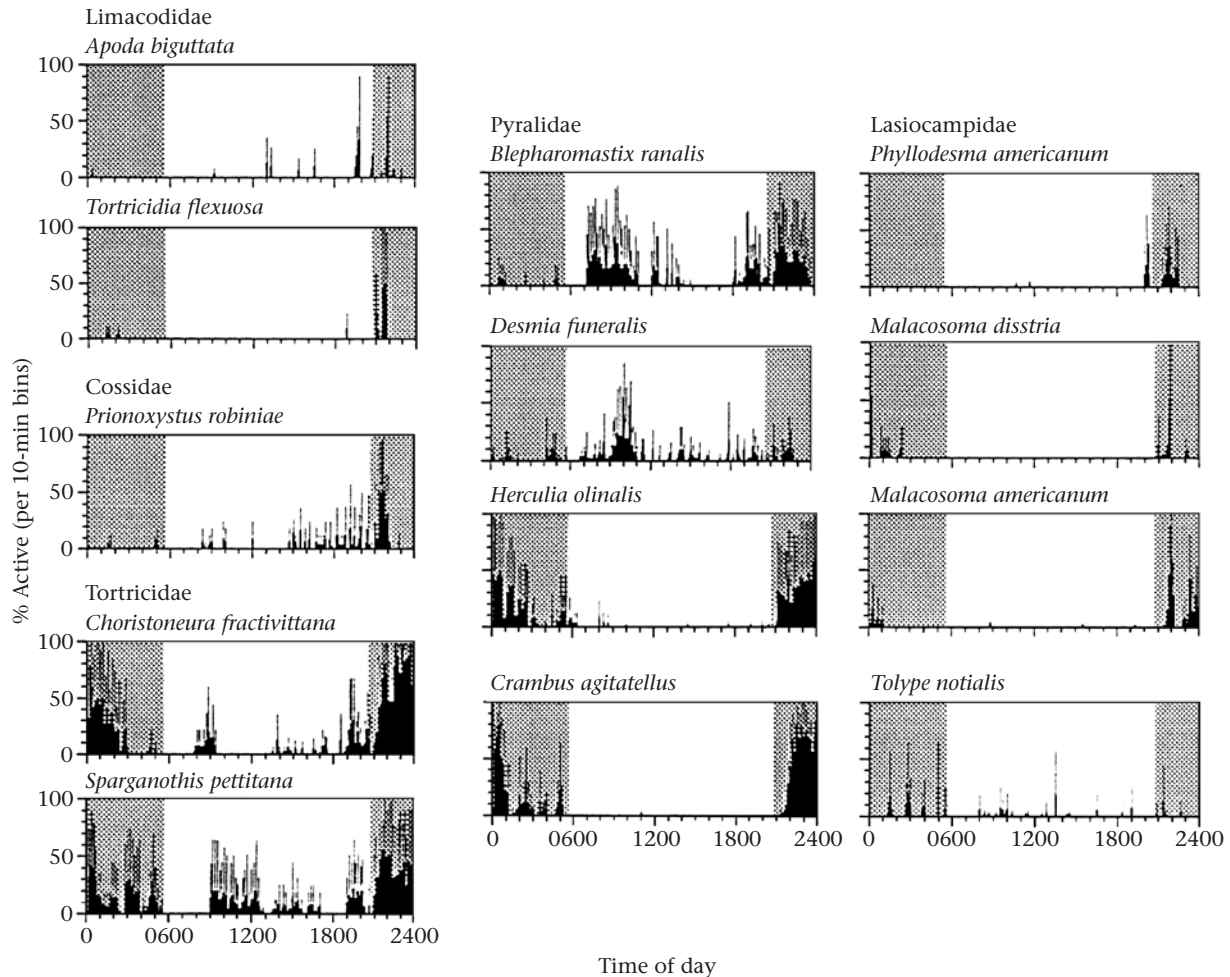
Figure 2. (a).

Figure 2. (a–g) Flight actograms of the earless (Limacodidae, Cossidae, Tortricidae, Lasiocampidae, Apatelodidae, Saturniidae, Sphingidae, Hesperiidae, Papilionidae, Pieridae, Lycaenidae and Nymphalidae) and eared (Pyralidae, Drepanidae, Geometridae, Notodontidae, Arctiidae, Lymantriidae and Noctuidae) lepidopteran families that were used in our study (sample sizes listed in Table 1). Bars represent means of 10 min flight times+one standard deviation. Shaded zones represent the mean summer periods of night as defined by the end of twilight until 40 min past sunrise, although individual DFPs were computed using the specific times on the dates when the actograms were measured.

(ca. 2200 hours), when exclusively nocturnal insects were active, and during the photophase (ca. 1030 hours), when little flight activity was observed. Similarly, for exclusively diurnal species, increasing flight appeared to correspond with rising temperatures from ca. 0700 to 1600 hours, when mean temperatures reach their maximum. However, as with nocturnal species, there was little flying observed in these species at equivalent temperatures in the night at ca. 2000 hours compared with their activity at 1100 hours.

Ears and DFP

As species are unlikely to represent phylogenetically independent data samples (Ridley 1989), we analysed DFP values at the superfamily level, which has been ordered phylogenetically (Robbins 1987; Kristensen & Skalski 1998). For these comparisons, we used only families with greater than one species and we averaged genera

with more than one species to provide a single value. Figure 4a, b describes the nocturnality and diel diversity of eared and earless superfamilies; we included sphingids within the earless bombycoids because no representatives of the eared Choerocampina or Acherontiina (Roeder et al. 1970; Göpfert & Wasserthal 1999a, b) were tested in this study. Butterflies (papilionoids+hesperioids, all ultrasound-earless) were significantly less nocturnal (Kruskal–Wallis: H_9=41.9, $P<0.05$; Dunn's a posteriori tests: $P<0.05$) than both eared and earless moths (Fig. 4a: Drepanoidea, Bombycoidea and Noctuoidea) and showed significantly less diel diversity (Kruskal–Wallis: H_9=24.2, $P<0.05$; Dunn's a posteriori tests: $P<0.05$) than the eared Noctuoidea (Fig. 4b). Interestingly, although the earless Bombycoidea were as nocturnal as eared moths (e.g. Geometroidea), they showed as little diel diversity as the butterflies (e.g. they were as nocturnal as the butterflies were diurnal). Only the noctuoids showed more diel diversity than the butterflies (primarily due to the DFP
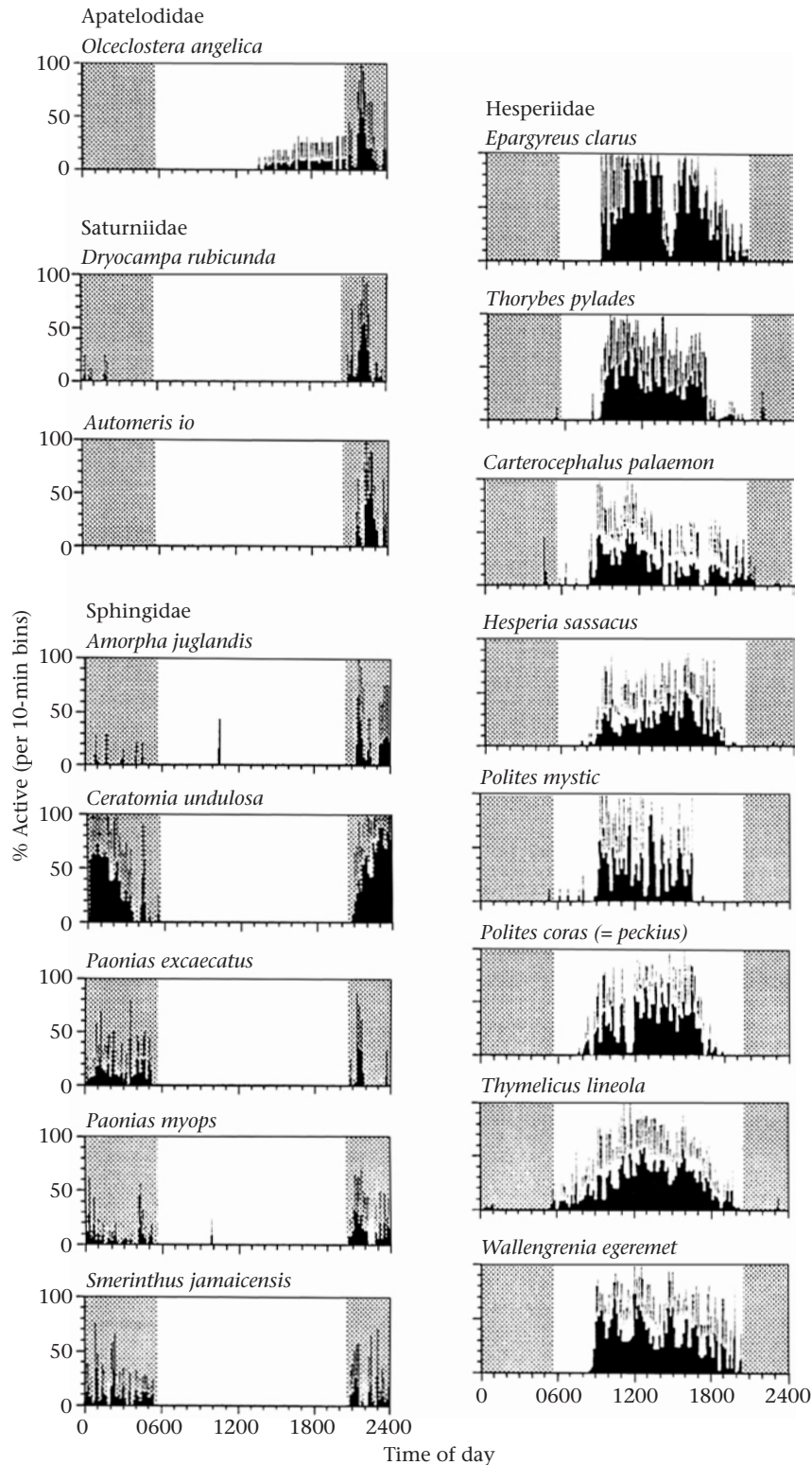
Figure 2. (b).

contributions of the unusually diurnal species in each of these clades). When all species were compared as a function of the number of auditory receptor neurons in each ears (Fig. 4c), eared Lepidoptera were more nocturnal (Kruskal–Wallis: H_9=20.0, $P<0.05$; Dunn's a posteriori tests: $P<0.05$) and more diel diverse (Kruskal–Wallis: H_9=19.1, $P<0.05$; Dunn's a posteriori tests: $P<0.05$) than earless species. However, there were no significant differences within the eared classes for either DFP value.
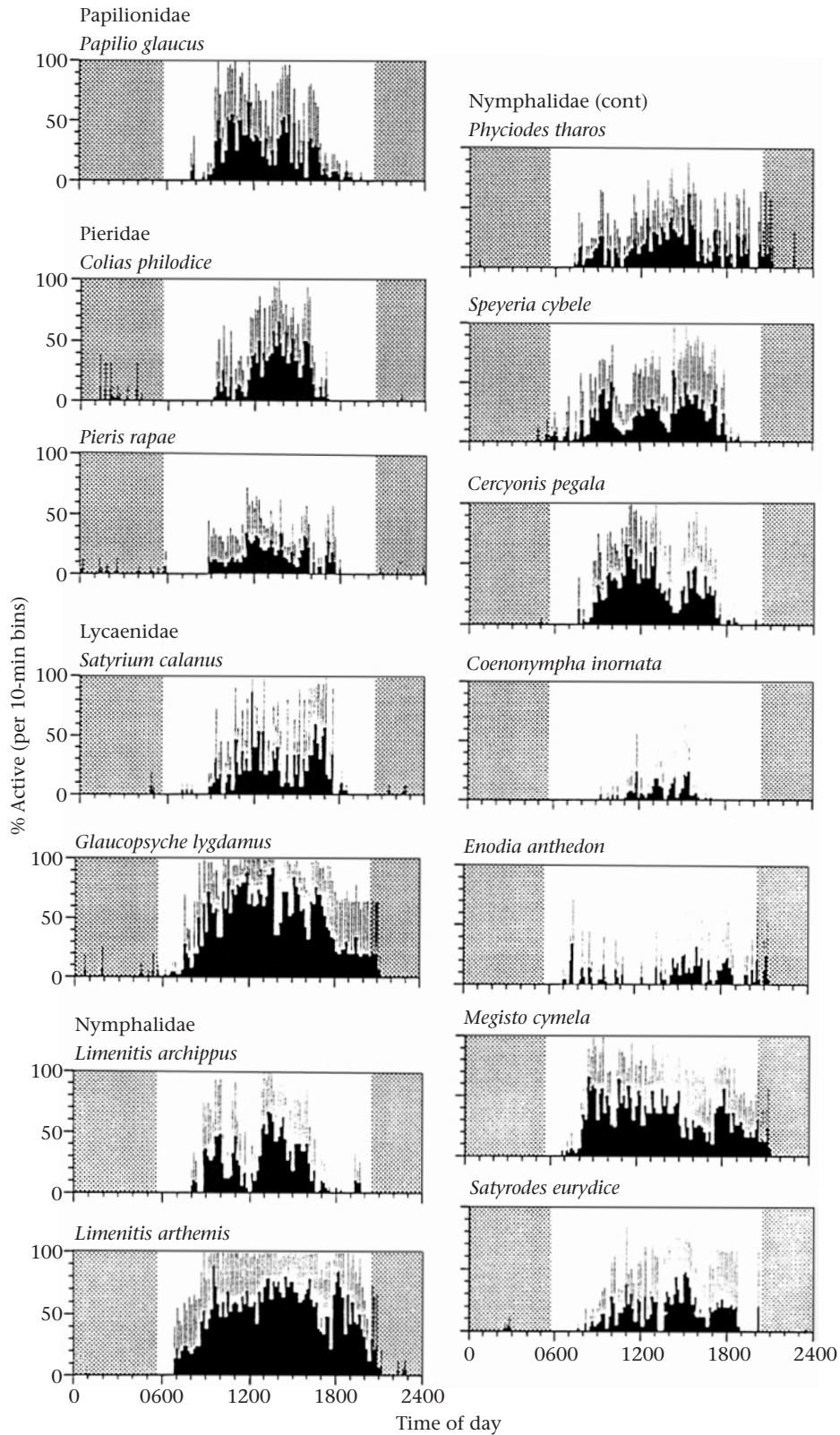
Figure 2. (c).

Phylogeny

In Fig. 5a, we have mapped our measured DFP characters of the taxa from our study onto the phylogenies of Kristensen & Skalski (1998) (superfamilies) and Weller et al. (1994) (noctuoid families) to produce an evolutionary description of day/night preferences in the
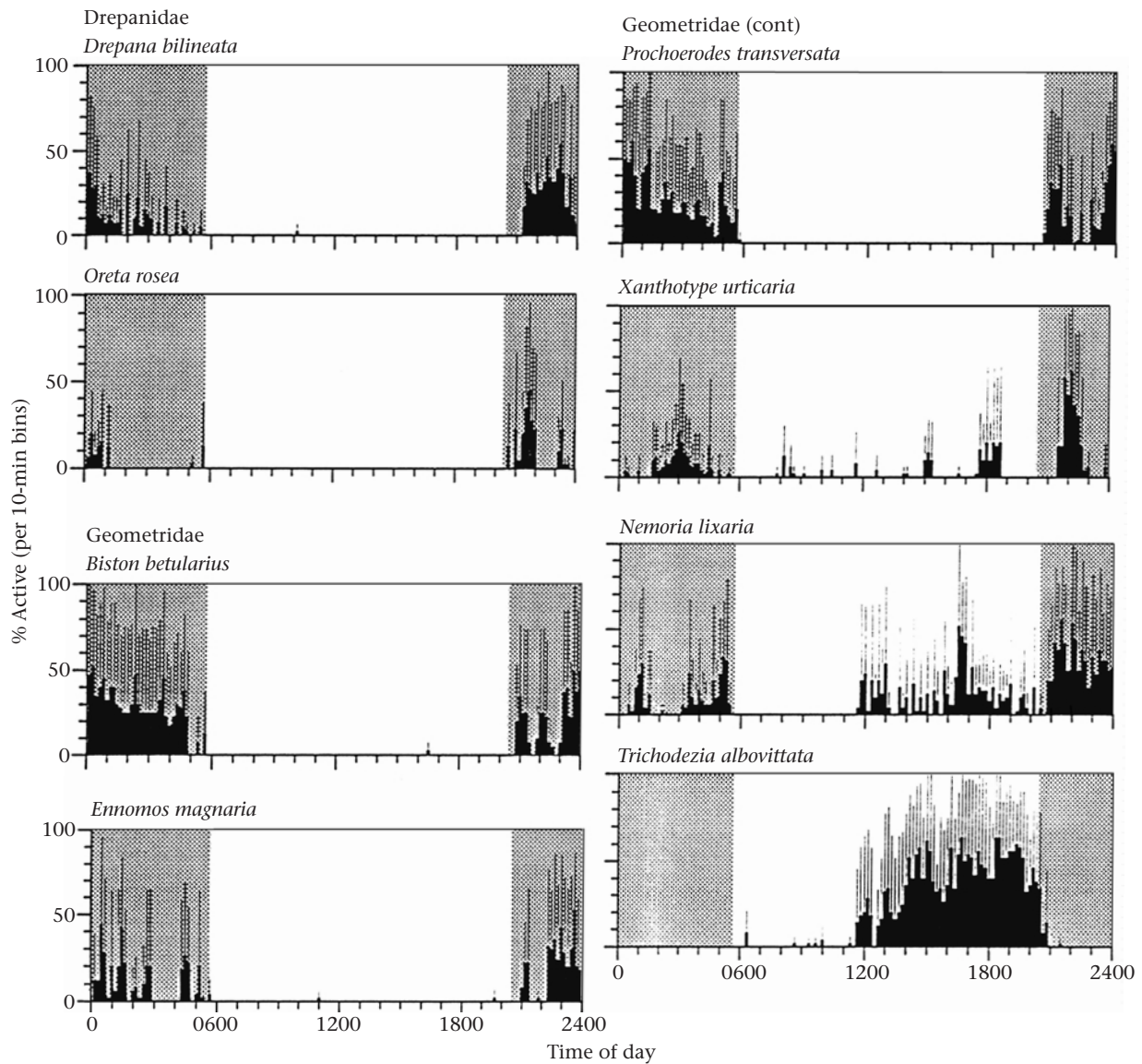
Figure 2. (d).

Macrolepidoptera (Fig. 5b). From these figures it appears that exclusive diurnality is apomorphic and appears only in the Papilionoidea and Hesperioidea (with the exception of one species), while exclusive nocturnality is phylogenetically unordered with only the Bombycoidea expressing a majority of nocturnal species. The Noctuoidea reveals clades with widely mixed DFPs ranging from exclusive nocturnality in the Notodontidae (with the exception of one species) to the highly diurnal Arctiidae (which contains the exclusively diurnal *Lycomorpha pholus*). Similarly, the Geometridae showed a high degree of exclusive nocturnality (also with another exclusively diurnal species, *Trichodezia albovittata*) but for all clades examined, exclusive nocturnality also appears to be an apomorphic trait. Mixed DFP was the most common trait of the species tested and, using the Zygaenoidea, Tortricoidea and Pyraloidea as outgroups, was the pleisiomorphic state for the Macrolepidoptera.

DISCUSSION

The first question that our study addresses is in regard to the definitions of 'diurnal' and 'nocturnal', diel periods traditionally set by abiotic criteria such as temperature or light levels (e.g. the 1985 *Oxford Dictionary of Current English* defines night as 'the time from sunset to sunrise'). Figure 3 illustrates that equivalent temperatures do not elicit equivalent levels of flight activity in either nocturnal or diurnal species, demonstrating that temperature alone does not determine the day versus night timing of flight preferences (cf. Quiring 1994). Delisle et al. (1998) demonstrated how the peak activity periods of the Nearctic moth *Lambdina fiscellaria* can be altered by changes in ambient temperatures within its scotophase, but these shifts do not change the overall diel preference of this species. Activity shifts are seen in other species as a function of various endogenous factors such as age or
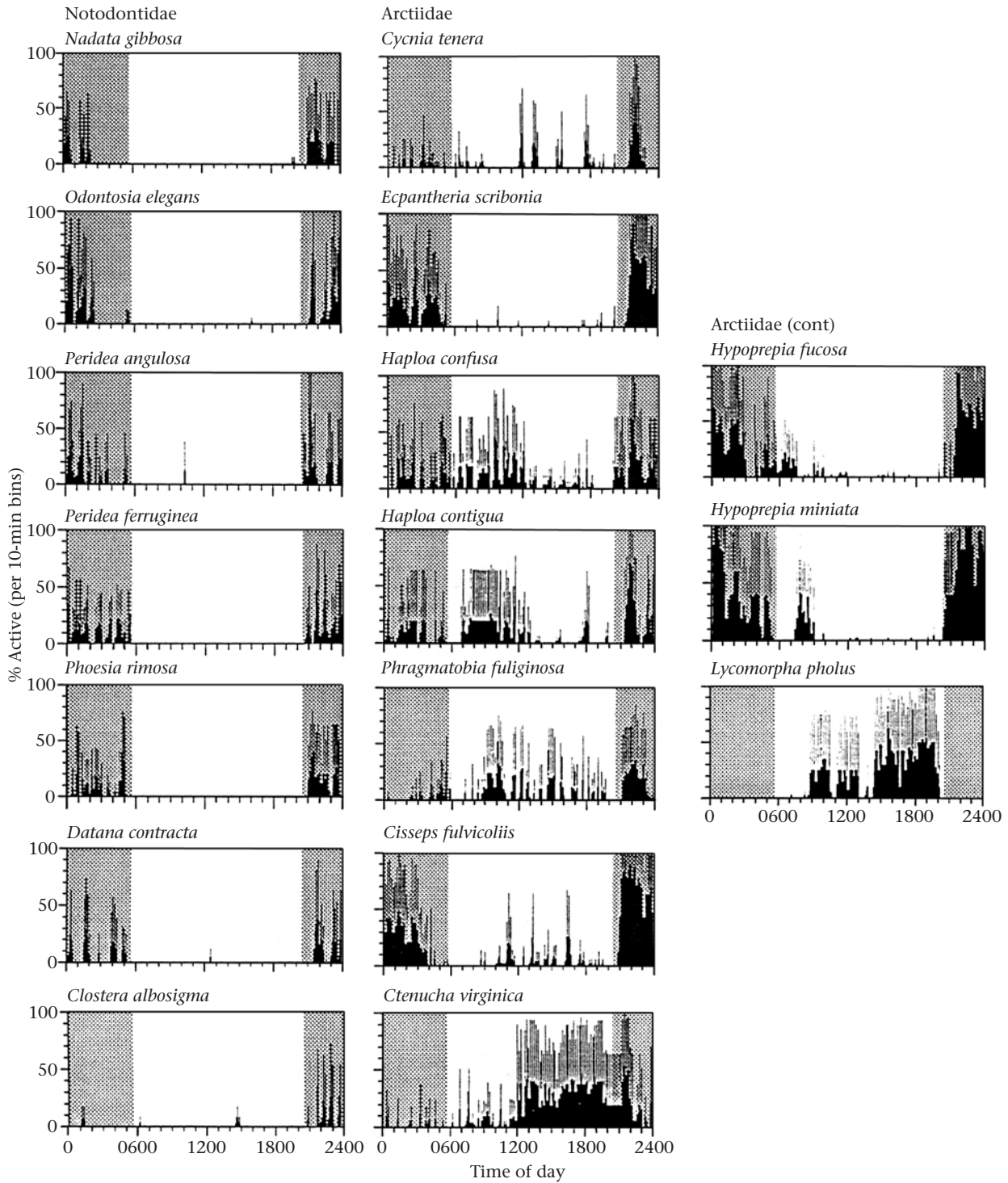
Figure 2. (e).

mating status and exogenous factors such as day length or light intensity (McNeil 1991) but these rarely result in changes from nocturnal to diurnal diel periodicity or vice versa. In moths, the dorsal ocelli have been implicated in the initiation of flight (Eaton et al. 1983), with lowering light levels acting to trigger the onset of nocturnal flying (Larsen 1943), and it appears that once activity has commenced it is correlated with temperature (e.g. Fig.

3b). We believe that, although physical stimuli provide exogenous cues for modifying the diel activities in Lepidoptera, their diel flight preferences originally evolved in response to the foraging behaviours and DFPs of their potential predators.

We propose that flighted predators have played a major role in determining the diel flight preferences of Lepidoptera (and perhaps all insects) and that ' diurnal ' is
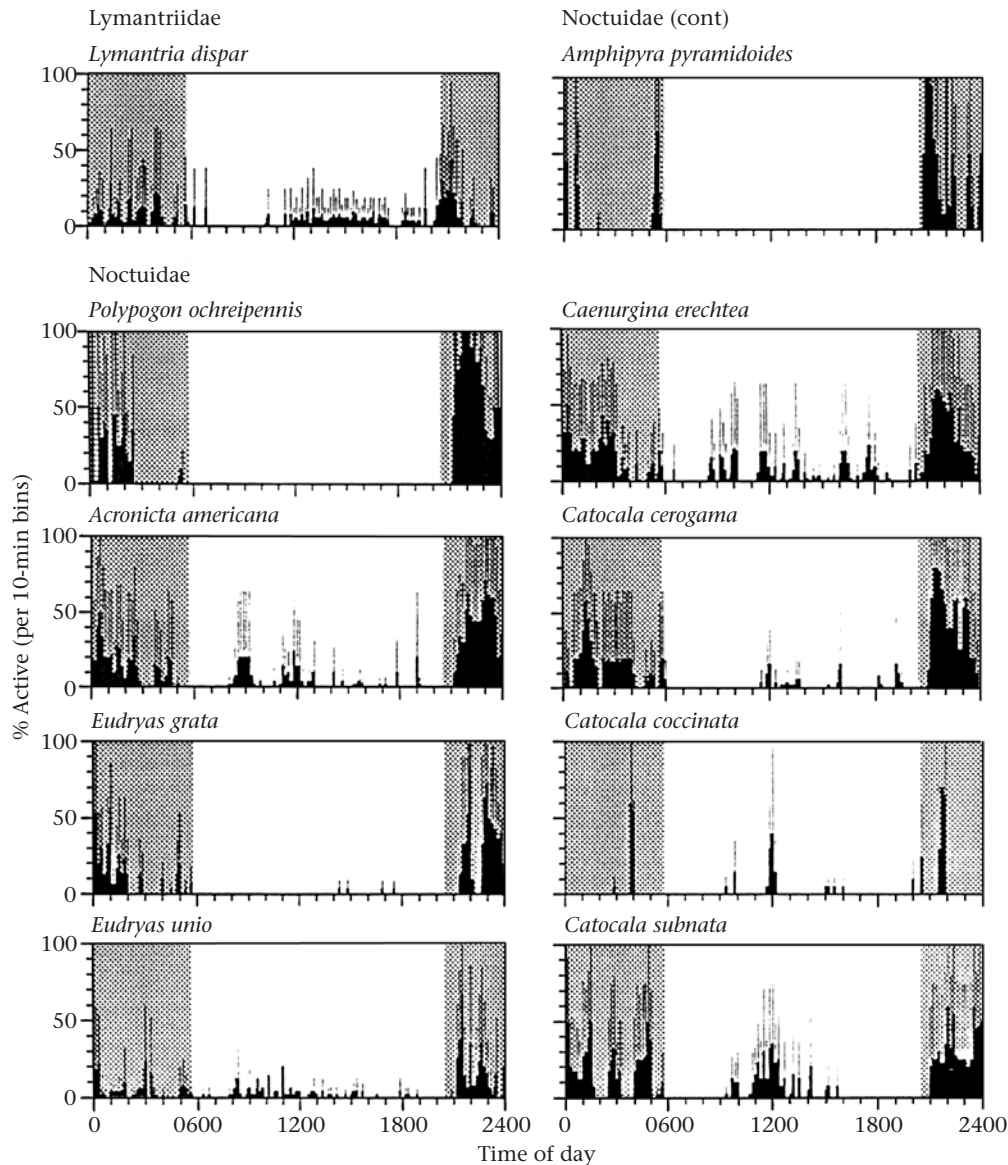
Figure 2. (f).

better understood as the period of selection pressure arising from predators such as birds, whereas 'nocturnal' is that period during which bats hunt. While the influence of birds as significant predators on insects is accepted (Pinheiro 1996), the same may not be held for insectivorous bats, whose nightly activities are less apparent to the casual observer. Bats are common and voracious insectivores in all forested regions of the world with the exception of certain Pacific islands (Fullard 1994, 2000) and constitute a powerful selective force on night-active insects even in habitats where their communities consist of only a single species (Belwood & Fullard 1984; Fullard 1984). That bats present a major problem for night-flight is further witnessed by the diversity of insects that possess auditory defences against these nocturnal predators (Surlykke 1988; Hoy & Robert 1996; Yager 1999) and in the degeneration or complete loss of ears

when bats are no longer a threat (Clarke 1971; Fullard et al. 1997; Surlykke et al. 1998; but see Rydell et al. 2000).

Auditory Adaptations to DFP

For Lepidoptera, ears provide an effective defence (Roeder & Treat 1962; Acharya & Fenton 1999) that allows for prolonged nocturnal flight at heights where bats hunt (Morrill & Fullard 1992; Lewis et al. 1993). Ultrasound-eared species should have greater combined day and night flight (diel diversity) because they possess both visual and auditory-based defences, allowing them to cope with both diurnal and nocturnal predators. Our comparisons of nocturnality and diel diversity (Fig. 4), however, indicates that auditory capability does not neatly explain lepidopteran DFP. Whereas it is evident
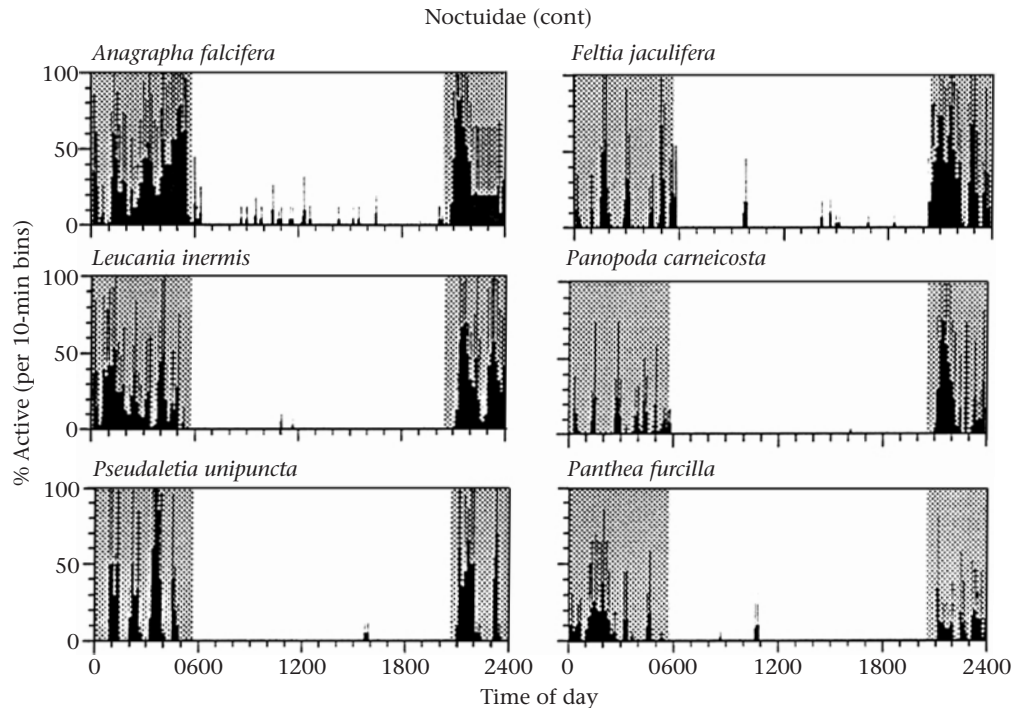
Figure 2. (g).

that the earless butterflies (Papilionoidea+Hesperioidea) are exclusively diurnal, the eared superfamilies, Pyraloidea, Geometroidea and Drepanoidea, similarly show little flight diversity (i.e. they are as nocturnal as butterflies are diurnal). Although comparisons of all species we tested revealed higher diel diversity in most eared taxa (Fig. 4c), this tendency did not hold for the single-celled ear species of the Notodontidae. A problem with these analyses remains the relatively poor phylogenetic resolution of this large and speciose insect order with the result that superfamily analyses (the main lepidopteran level currently resolved) obscures the alternative antipredator strategies that certain species have taken that are contrary to their expected DFPs. For example, many species of arctiids employ aposematic coloration as a visually based defence, which may have favoured an increased use of the photophase (Dreisig 1986). The evolution of these Lepidoptera with their food plants and resultant chemical defences (Weller et al. 1999) will introduce variance into the relationships between ears and DFPs.

Why Are Butterflies Diurnal?

The Rhopalocera consists of the ultrasound-earless Papilionoidea (true butterflies) and Hesperioidea (skipper butterflies) plus the Hedyloidea, of which some species have been described as eared (Scoble 1992; Yack & Fullard 2000). These three superfamilies appear to represent (at least tentatively; Kristensen & Skaski 1998) a monophyletic clade, and our study quantitatively demonstrates that two of these butterfly superfamilies are exclusively day-flying insects. That the Papilionoidea and Hesperioidea (with one species exception) in our study were the

only superfamilies with exclusively diurnal flight suggests that a common characteristic shared by these clades has resulted in their evolved DFPs. Fullard (1988) hypothesized that butterflies, lacking ultrasound-sensitive ears, are diurnal because of echolocating bats and the present study now provides empirical evidence supporting this hypothesis in the Papilionoidea and the Hesperioidea. Which evolved first for these butterflies, diurnal flight (Morrill & Fullard 1992) or bats (Fullard 1998; Yack & Fullard 2000), remains an unanswered question. We suggest that the exclusive diurnality of papilionoid butterflies in other locations (e.g. Venezuela: Fullard et al. 2000; Polynesia and Australia: Fullard 2000), combined with their extravagant diversity of visual designs indicates that at least this butterfly clade had adopted diurnal flight before echolocating bats appeared. Although visual system adaptations (e.g. appositional eyes) may now act as constraints keeping butterflies diurnal (Fullard 2000), other insects appear to have overcome these restrictions to exploit the advantages of night-flight (e.g. honeybees: Warrant et al. 1996). The report of ultrasound responsiveness in one species of a nocturnally flighted hedyloid (Yack & Fullard 2000) suggests that this unusual group of butterflies has either retained or reversed to a DFP of nocturnal flight (Aiello 1992) because of their eared condition.

Why Are Earless Moths Nocturnal?

Our results indicate that the earless Bombycoidea are as nocturnal as eared superfamilies (Fig. 4a) even though they face the threat of bats without auditory defences. In light of the fact that the earless butterflies are diurnal,
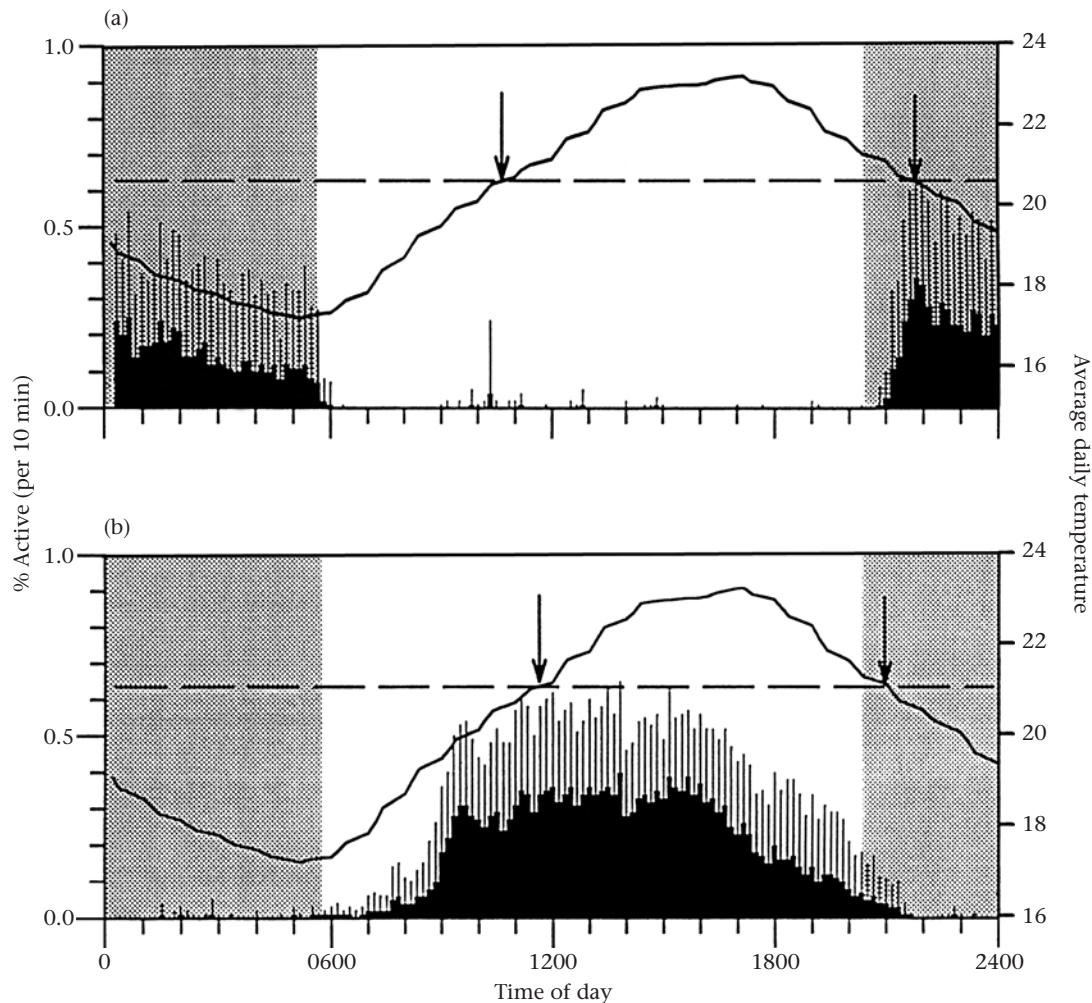
Figure 3. The average DFP ($\bar{X}\pm$SD) of all species that were (a) greater than 90% or (b) less than 10% nocturnal is plotted against the average temperature sampled every 20 min throughout the summer. Arrows indicate times of day and night where temperatures were equal (dashed line) but flight activity was not.

why have earless moths followed an opposite evolutionary path? We suggest that the sporadic and ground-hugging flight of certain earless moths (Morrill & Fullard 1992; Lewis et al. 1993; Rydell 1998), while providing protection against bats, would be a behavioural liability for avoiding visually hunting predators during the day. Also, earless moths are generally larger and heavier, traits that may protect them against smaller species of mouth-echolocating bats (Barclay & Brigham 1994) but would be less effective against birds. Once earless moths adopted exclusive nocturnality, visual adaptations may have evolved that further constrained these Lepidoptera from day-flight (e.g. mosquitoes: Land et al. 1999). Butterflies, excluded from night-flight by bats, may have already possessed rapid flight and could have used this pre-adaptation to escape predacious birds (Pinheiro 1996). There are two lepidopteran families that are at odds with this theory. Prominent moths (Notodontidae) are eared (Surlykke 1984; Fullard et al. 1998) but also displayed one of the highest levels of exclusive nocturnality (i.e. low diel diversity) measured. That the ears of notodontids are among the least sensitive of all moths suggests that these

Lepidoptera may be less able to use auditory defences and thus rely on reduced flight as a nonauditory defence resulting in nocturnal DFPs. On the other hand, hawk-moths (Sphingidae) are also primarily nocturnal and earless (but see Göpfert & Wasserthal 1999a, b) but, unlike saturniids, are rapid, high-altitude fliers (Lewis et al. 1993). Rydell & Lancaster (2000) suggest that earless-ness in the Lepidoptera has favoured greater body size and thoracic temperatures, allowing for high flight speeds that would provide for an effective nonauditory defence against bats. Although our actograms did not measure it, sphingids may employ short bursts of rapid flight, which would allow them to travel long distances while keeping their total nightly conspicuousness to bats low.

Evolution of Diel Flight Periodicity in the Macrolepidoptera

Our phylogenetic analysis of day/night preferences shown in Fig. 5b revealed some unexpected results. The first being that exclusive nocturnality is neither a
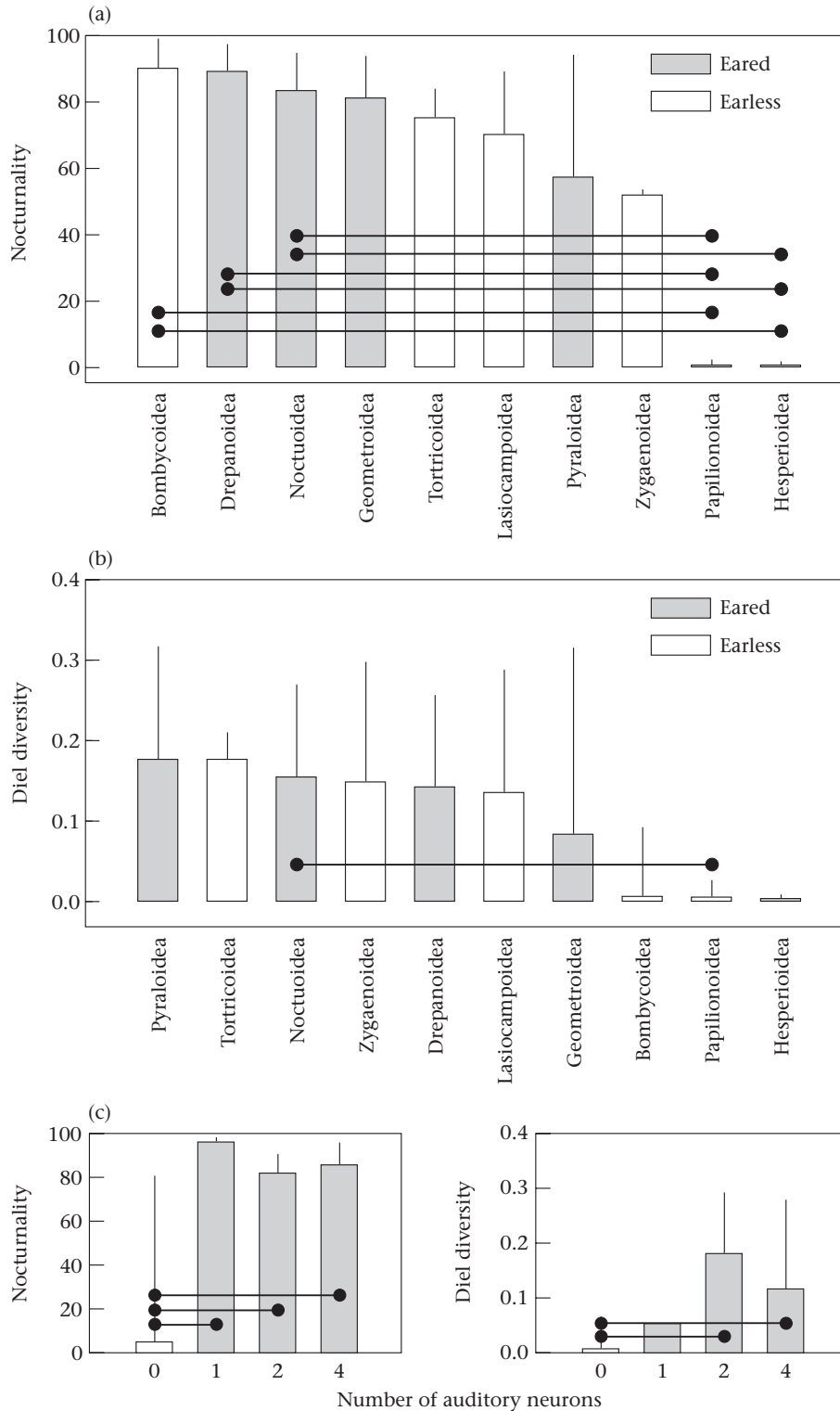
Figure 4. The median (+75th percentile) nocturnality (a) and diel diversity (b) values of superfamilies containing greater than one species and their auditory conditions (all bombycoids, including sphingids, and papilionoids are assumed earless). (c) Multiple comparisons of all species (medians+75th percentile) nocturnality and diel diversity values as a function of the number of auditory receptor cells per ear. Significant differences (Kruskal-Wallis and Dunn's a posteriori multiple comparison tests: $P<0.05$) are indicated with connecting lines.

pleisiomorphic trait nor a widespread trait within the Macrolepidoptera. Figure 5b suggests that these insects originally displayed mixed day/night flight activity and

that exclusive nocturnality is an apomorphic trait. Even within taxa whose median DFPs indicate exclusive nocturnality (e.g. Sphingidae), there were species with mixed
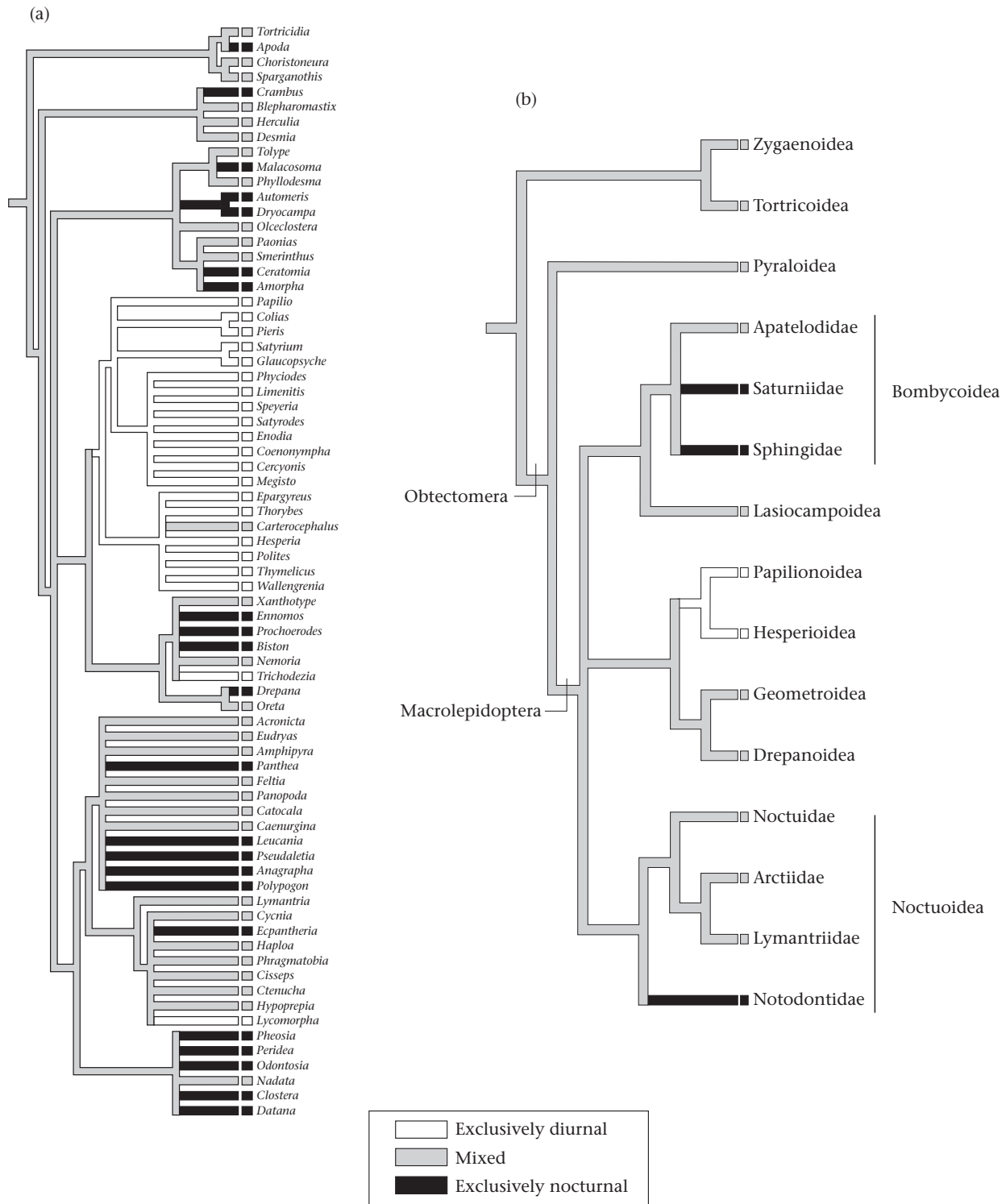
Figure 5. (a) Our map of the nocturnal flight activity of the genera represented in our study and its projection onto (b) the proposed phylogenies of lepidopteran superfamilies (Kristensen & Skalski 1998) and noctuoid families (Weller et al. 1994).

diel preferences. The value of light and vision may have always been great enough for these insects to have favoured some use of the day hours, a theory supported by the fact that there are no lepidopteran eyes as degenerate as those of invertebrates that have entered completely unlit niches (e.g. cave-dwelling amphipods: Jones & Culver 1989). The second trend seen in Fig. 5b is the rarity of exclusive diurnality within the Lepidoptera. Only the Papilionoidea and Hesperioidea (with one exception) were exclusively diurnal and reports of nocturnal flight in the Costa Rican skipper, *Celaenorrhinus fritzgairtneri* (DeVries et al. 1987) suggest that mixed DFP
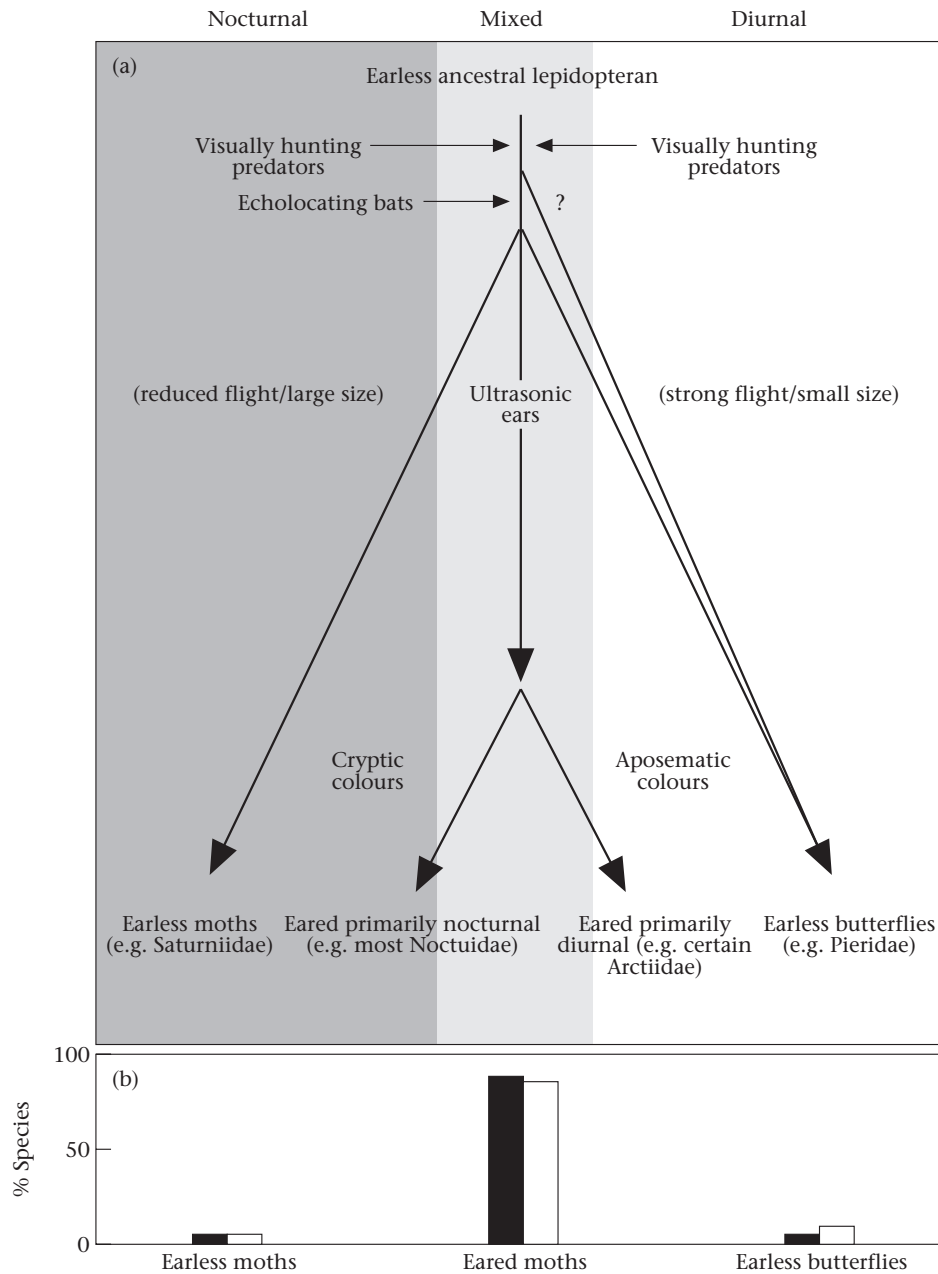
Figure 6. (a) A proposed evolution of DFP and sensory defensive adaptations in the Lepidoptera (see Discussion). The question of the point of divergence of the diurnal butterflies ('?') is discussed in the text. (b) A comparison of the auditory classes of Lepidoptera (i.e. the percentage of species in each class) examined in this study at two locations: Australia (■; Nielsen & Common 1991) and Ontario (□; Riotte 1992).

may be more common than currently believed in the hesperioid superfamily.

Figure 6a describes the evolution of DFP and sensory defences suggested by our results. Prior to the appearance of echolocating bats, the combined predation pressures of visually hunting diurnal and nocturnal predators selected for Lepidoptera with mixed diel flight preferences. With only the need for vision as a defence, these insects could maximize their reproductive flight time by choosing both day and night hours in which to fly, or alternatively, to switch between exclusive diurnal and nocturnal flight. Although Fig. 6a suggests that prior to the appearance of echolocating bats, Lepidoptera may have expressed an equally mixed DFP, it is more likely that the preponderance of diurnal predators (birds) would have favoured mixed, primarily nocturnal DFPs, a suggestion borne out by the fact that greatest number of species (42.1%) in our study displayed this flight preference.

The appearance of echolocating bats brought about a new selective force that greatly increased the potential costs of night flight and resulted in exclusive diurnality in the earless papilionoid and hesperioid butterflies and auditory-based defences for most other Lepidoptera (Fullard 1988). Once butterflies adopted day-flight, physiological and social constraints (e.g. the use of visual versus pheromonal mating signals), locked these insects

into their diurnal DFP. Nonauditory-based defences, while allowing earless moths to exist at night, appear to have constrained these insects into exclusive nocturnality. The evolution of ultrasound-sensitive ears, by countering the predation pressure of bats while maintaining effective visual defences against birds, has retained the pleisiomorphic state of mixed day and night flight, suggesting that these sensory and behavioural adaptations have resulted in eared moths with the greatest species diversity of the Lepidoptera (Fig. 6b).

Acknowledgments

References

Acharya, L. & Fenton, M. B. 1999. Bat attacks and moth defensive behaviour around street lights. *Canadian Journal of Zoology*, **77**, 27–33.

Acharya, L. & McNeil, J. N. 1998. Predation risk and mating behaviour: the responses of moths to bat-like ultrasound. *Behavioral Ecology*, **9**, 552–558.

Aiello, A. 1992. Nocturnal butterflies in Panama, Hedylidae (Lepidoptera: Rhopalocera). In: *Insects of Panama and Meso-america* (Ed. by D. Quintero & A. Aiello), pp. 549–553. New York: Oxford Science.

Arikawa, K., Mizuno, S., Scholten, D. G. W., Kinoshita, M. T., Kitamoto, J. & Stavenga, D. G. 1999. An ultraviolet absorbing pigment causes a narrow-band violet receptor and a single-peaked green receptor in the eye of the butterfly *Papilio*. *Vision Research*, **39**, 1–8.

Barclay, R. M. R. 1982. Interindividual use of echolocation calls: eavesdropping by bats. *Behavioral Ecology and Sociobiology*, **10**, 271–275.

Barclay, R. M. R. & Brigham, R. M. 1994. Constraints on optimal foraging: a field-test of prey discrimination by echolocating insectivorous bats. *Animal Behaviour*, **48**, 1013–1021.

Belwood, J. J. & Fenton, M. B. 1976. Variation in the diet of *Myotis lucifugus* (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology*, **54**, 1674–1678.

Belwood, J. J. & Fullard, J. H. 1984. Echolocation and foraging behaviour in the Hawaiian hoary bat (*Lasiurus cinereus semotus*). *Canadian Journal of Zoology*, **62**, 2113–2120.

Cardé, R. T., Doane, C. C. & Roelofs, W. L. 1974. Diel periodicity of male sex pheromone response and female attractiveness in the gypsy moth (Lepidoptera: Lymantriidae). *Canadian Entomologist*, **106**, 479–484.

Chinery, M. 1989. *Butterflies and Day-Flying Moths of Britain and Europe*. Glasgow: William Collins.

Clarke, J. F. G. 1971. The Lepidoptera of Rapa Island. *Smithsonian Contributions to Zoology*, **56**, 1–282.

Conner, W. E. 1987. Ultrasound: its role in the courtship of the arctiid moth, *Cycnia tenera*. *Naturwissenschaften*, **43**, 1029–1031.

Conner, W. E. & Masters, W. M. 1978. Infrared video viewing. *Science*, **199**, 1004.

Covell, C. V., Jr. 1984. *A Field Guide to the Moths of Eastern North America*. New York: Houghton Mifflin.

Cutler, D. E., Bennett, R. R., Stevenson, R. D. & White, R. H. 1995. Feeding behavior in the nocturnal moth *Manduca sexta* is mediated mainly in the blue receptors but where are they located in the retina? *Journal of Experimental Biology*, **198**, 1909–1917.

Delisle, J., West, R. J. & Bowers, W. W. 1998. The relative performance of pheromone and light traps in monitoring the seasonal activity of both sexes of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria*. *Entomologia Experimentalis et Applicata*, **89**, 87–98.

DeVries, P. J., Schull, J. & Greig, N. 1987. Synchronous nocturnal activity and gregarious roosting in the neotropical skipper butterfly *Celaenorrhinus fritzgaertneri* (Lepidoptera: Hesperiidae). *Zoological Journal of the Linnaean Society*, **89**, 89–103.

Dreisig, H. 1986. Timing of daily activities in adult Lepidoptera. *Entomologia Generalis*, **12**, 25–43.

Eaton, J. L., Tignor, K. R. & Holtzman, G. I. 1983. Role of moth ocelli in timing flight initiation at dusk. *Physiological Entomology*, **8**, 371–375.

Edmunds, H. 1974. *Defense in Animals*. New York: Longman.

Edwards, D. K. 1962. Laboratory determinations of the daily flight times of separate sexes of some moths in naturally changing light. *Canadian Journal of Zoology*, **40**, 511–530.

Eggers, F. 1919. Das thoracale bitympanale Organ einer Gruppe der Lepidoptera Heterocera. *Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere*, **41**, 273–376.

Ehnbom, K. 1948. Studies on the central and sympathetic nervous system and some sense organs in the head of neuropteroid insects. *Opuscula Entomologica Supplementum*, **8**, 1–162.

Fleishman, L. J. 1986. Motion detection in the presence and absence of background motion in an *Anolis* lizard. *Journal of Comparative Physiology A*, **159**, 711–720.

Forbes, W. T. M. 1960. *Lepidoptera of New York and Neighboring States*. Part IV. New York: New York State College Agricultural Experiment Station, Memoir 371.

Fullard, J. H. 1984. Acoustic relationships between tympanate moths and the Hawaiian hoary bat (*Lasiurus cinereus semotus*). *Journal of Comparative Physiology A*, **155**, 795–801.

Fullard, J. H. 1988. The tuning of moth ears. *Experientia*, **44**, 423–428.

Fullard, J. H. 1994. Auditory changes in moths endemic to a bat-free habitat. *Journal of Evolutionary Biology*, **7**, 435–445.

Fullard, J. H. 1998. Sensory coevolution of moths and bats. In: *Comparative Hearing: Insects* (Ed. by R. R. Hoy, A. N. Popper & R. R. Fay), pp. 279–326. New York: Springer.

Fullard, J. H. 2000. Day-flying butterflies remain day-flying in a Polynesian, bat-free habitat. *Proceedings of the Royal Society of London, Series B*, **267**, 2295–2300.

Fullard, J. H. & Dawson, J. W. 1999. Why do diurnal moths have ears? *Naturwissenschaften*, **86**, 276–279.

Fullard, J. H., Dawson, J. W., Otero, L. D. & Surlykke, A. 1997. Bat-deafness in day-flying moths (Lepidoptera, Notodontidae, Dioptinae). *Journal of Comparative Physiology A*, **181**, 477–483.

Fullard, J. H., Forrest, E. & Surlykke, A. 1998. Intensity responses of the single auditory receptor of notodontid moths: a test of the peripheral interaction hypothesis in moth ears. *Journal of Experimental Biology*, **201**, 3419–3424.

Fullard, J. H., Otero, L. D., Orellana, A. & Surlykke, A. 2000. Auditory sensitivity and diel flight activity in neotropical Lepidoptera. *Annals of the Entomological Society of America*, **93**, 956–965.

Göpfert, M. C. & Wasserthal, L. T. 1999. Hearing with the mouthparts: behavioural responses and the structural basis of

ultrasound perception in acherontiine hawkmoths. *Journal of Experimental Biology*, **202**, 909–918.

Göpfert, M. C. & Wasserthal, L. T. 1999. Auditory sensory cells in hawkmoths: identification, physiology and structure. *Journal of Experimental Biology*, **202**, 1579–1587.

Horridge, G. A., Mclean, M., Stange, G. & Lillywhite, P. G. 1977. A diurnal moth superposition eye with high resolution *Phalaenoides tristifica* (Agaristidae). *Proceedings of the Royal Society of London, Series B*, **196**, 233–250.

Hoy, R. R. & Robert, D. 1996. Tympanal hearing in insects. *Annual Review of Entomology*, **41**, 433–450.

Jones, R. & Culver, D. C. 1989. Evidence for selection on sensory structures in a cave population of *Gammarus minus* (Amphipoda). *Evolution*, **43**, 688–693.

Kreusel, B. & Häuser, C. L. 1997. Relationships between tympanal organ structure and activity patterns in ctenuchine moths (Lepidoptera, Arctiidae). *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **90**, 176.

Kristensen, N. P. & Skalski, A. W. 1998. Palaeontology and phylogeny. In: *Lepidoptera: Moths and Butterflies. Handbuch der Zoologie* (Ed. by N. P. Kristensen), pp. 7–25. New York: de Gruyter.

Land, M. F., Gibson, G., Horwood, J. & Zeil, J. 1999. Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *Journal of Comparative Physiology A*, **185**, 91–103.

Larsen, E. B. 1943. The importance of master factors for the activity of noctuids. *Entomologiske Meddelelser*, **23**, 352–374.

Lewis, F., Fullard, J. H. & Morrill, S. 1993. Auditory influences on the flight behaviour of moths at a Nearctic site. II. Flight times, heights and erraticism. *Canadian Journal of Zoology*, **71**, 1562–1568.

McNeil, J. N. 1991. Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Annual Review of Entomology*, **36**, 407–430.

Maddison, W. P. & Maddison, D. R. 1992. *MacClade. Analysis of Phylogeny and Character Evolution. Version 3*. Sunderland, Massachusetts: Sinauer.

Monge-Nájera, J., Hernández, F., González, M. I., Soley, J., Araya, J. & Zolla, S. 1998. Spatial distribution, territoriality and sound production by tropical cryptic butterflies (*Hamadryas*, Lepidoptera: Nymphalidae): implications for the 'industrial melanism' debate. *Revista de Biologica Tropical*, **46**, 297–330.

Morrill, S. B. & Fullard, J. H. 1992. Auditory influences on the flight behaviour of moths in a Nearctic site. I. Flight tendency. *Canadian Journal of Zoology*, **70**, 1097–1101.

Nielsen, E. S. & Common, I. F. B. 1991. Lepidoptera. In: *The Insects of Australia*. 2nd edn (Ed. by I. D. Naumann), pp. 817–915. Melbourne, Australia: Melbourne University Press.

Opler, P. A. 1998. *A Field Guide to the Eastern Butterflies*. New York: Houghton Mifflin.

Otero, L. D. 1990. Estudio de algunos caracteres para si uso en la clasificación de Eurytelinae (Lepidoptera: Nymphalidae). *Boletín de Entomología Venezolana (Nueva Serie)*, **5**, 123–138.

Pinheiro, C. E. G. 1996. Palatablility and escaping ability in Neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biological Journal of the Linnean Society*, **59**, 351–365.

Powell, J. A. 1973. A systematic monograph of New World ethmiid moths (Lepidoptera: Gelechioidea). *Smithsonian Contributions to Zoology*, **120**, 1–302.

Quiring, D. T. 1994. Diel activity patterns of a nocturnal moth, *Zeiraphera canadensis*, in nature. *Entomologia Experimentalis et Applicata*, **73**, 111–120.

Ribarič, D. & Gogala, M. 1996. Acoustic behaviour of some butterfly species of the genus *Erebia* (Lepidoptera: Satyridae). *Acta Entomologica Slovenica*, **4**, 5–12.

Ridley, M. 1989. Why not to use species in comparative tests. *Journal of Theoretical Biology*, **136**, 361–364.

Riley, J. R., Smith, A. D. & Bettany, B. W. 1990. The use of video equipment to record in three dimensions the flight trajectories of *Heliothis armigera* and other noctuids at night. *Physiological Entomology*, **15**, 73–80.

Riley, J. R., Armes, N. J., Reynolds, D. R. & Smith, A. D. 1992. Nocturnal observations on the emergence and flight behaviour of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in the post-rainy season in central India. *Bulletin of Entomological Research*, **82**, 243–256.

Riotte, J. C. E. 1992. *Annotated List of Ontario Lepidoptera. Miscellaneous Publications in the Life Sciences*. Toronto: Royal Ontario Museum.

Robbins, C. S. 1981. Effect of time of day on bird activity. In: *Studies in Avian Biology No. 6* (Ed. by C. J. Ralph & J. M. Scott), pp. 275–286. Lawrence, Kansas: Allen Press.

Robbins, R. K. 1987. Logic and phylogeny: a critique of Scott's phylogenies to the butterflies and Macrolepidoptera. *Journal of the Lepidopterist's Society*, **41**, 214–216.

Roeder, K. D. 1967. *Nerve Cells and Insect Behavior*. Cambridge, Massachusetts: Harvard University Press.

Roeder, K. D. 1974. Acoustic sensory responses and possible bat-evasion tactics of certain moths. In: *Proceedings of the Canadian Society of Zoologists Annual Meeting* (Ed. by M. D. B. Burt), pp. 71–78. Fredericton: University of New Brunswick.

Roeder, K. D. & Treat, A. E. 1962. The acoustic detection of bats by moths. *Verhandlungen: XI. Internationaler Kongress für Entomologie*, **3**, 7–11.

Roeder, K. D., Treat, A. E. & Van de Berg, J. S. 1970. Distal lobe of the pilifer: an ultrasonic receptor in choerocampine hawkmoths. *Science*, **170**, 1098–1099.

Rydell, J. 1998. Bat defence in lekking ghost moths (*Hepialus humuli*), a moth without ultrasonic hearing. *Proceedings of the Royal Society of London, Series B*, **265**, 1373–1376.

Rydell, J. & Lancaster, W. C. 2000. Flight and thermoregulation in moths shaped by predation from bats. *Oikos*, **88**, 13–18.

Rydell, J., Roininen, H. & Philip, K. W. 2000. Persistence of bat defence reactions in high Arctic moths (Lepidoptera). *Proceedings of the Royal Society of London, Series B*, **267**, 553–557.

Sakaluk, S. K. & Belwood, J. J. 1984. Gecko phonotaxis to cricket calling song: a case of satellite predation. *Animal Behaviour*, **32**, 659–662.

Scherer, C. & Kolb, G. 1987. Behavioral experiments on the visual processing of color stimuli in *Pieris* (Lepidoptera). *Journal of Comparative Physiology A*, **160**, 645–656.

Schmitz, H. & Bleckmann, H. 1998. The photomechanic infrared receptor for the detection of forest fires in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). *Journal of Comparative Physiology A*, **182**, 647–657.

Scoble, M. J. 1992. *The Lepidoptera*. Oxford: Oxford University Press.

Surlykke, A. 1984. Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *Journal of Experimental Biology*, **113**, 323–335.

Surlykke, A. 1988. Interaction between echolocating bats and their prey. In: *Animal Sonar* (Ed. by P. E. Nachtigall & P. W. B. Moore), pp. 551–567. New York: Plenum.

Surlykke, A., Skals, N., Rydell, J. & Svensson, M. 1998. Sonic hearing in a diurnal geometrid moth, *Archieris parthenias*, temporally isolated from bats. *Naturwissenschaften*, **85**, 36–37.

Svensson, M. 1996. Sexual selection in moths: the role of chemical communication. *Biological Reviews*, **71**, 113–135.

Svensson, M. G. E., Rydell, J. & Brown, R. 1999. Bat predation and flight timing of winter moths, *Epirrita* and *Operophtera* species (Lepidoptera, Geometridae). *Oikos*, **84**, 193–198.

Swihart, S. L. 1967. Hearing in butterflies (Nymphalidae: *Heliconius, Ageronia*). *Journal of Insect Physiology*, **13**, 469–476.

Ward, P. S., Harmsen, R. & Hebert, P. D. N. 1974. The Macroheterocera of south-eastern Ontario. *Journal of Research on the Lepidoptera*, **13**, 23–42.

Warrant, E., Porombka, T. & Kirchner, W. H. 1996. Neural image enhancement allows honeybees to see at night. *Proceedings of the Royal Society of London, Series B*, **263**, 1521–1525.

Webster, R. P. & Conner, W. E. 1986. Effects of temperature, photoperiod, and light intensity on the calling rhythm in arctiid moths. *Entomologia Experimentalis et Applicata*, **40**, 239–245.

Weller, S. J., Pashley, D. P., Martin, J. A. & Constable, J. J. 1994. Phylogeny of noctuoid moths and the utility of combining independent nuclear and mitochondrial genes. *Systematic Biology*, **43**, 194–211.

Weller, S. J., Jacobson, N. L. & Conner, W. E. 1999. The evolution of chemical defences and mating systems in tiger moths (Lepidoptera: Arctiidae). *Biological Journal of the Linnean Society*, **68**, 557–578.

Yack, J. E. & Fullard, J. H. 2000. Ultrasonic hearing in nocturnal butterflies. *Nature*, **403**, 265–266.

Yack, J. E., Otero, L. D., Dawson, J. W., Surlykke, A. & Fullard, J. H. 2000. Sound production and hearing in the blue cracker butterfly, *Hamadryas feronia* (Lepidoptera, Nymphalidae) from Venezuela. *Journal of Experimental Biology*, **203**, 3689–3702.

Yager, D. D. 1999. Structure, development, and evolution of insect auditory systems. *Microscopy Research and Technique*, **47**, 380–400.