

Phenotypic plasticity of flying-fox reproduction aligns the genome-encoded rhythm to environmental conditions

G.M. O'Brien

Human Biology and Physiology, University of New England, NSW, 2351, Australia. gobrien@une.edu.au

ABSTRACT

Timing of seasonal reproduction by flying-foxes is apparently dependent on an endogenous circannual rhythm, coded in their genome. Ultimately, evolution of an endogenous rhythm aligns an important reproductive stage, such as lactation, with resource availability such as the occurrence of maximum plant productivity; animals then do not have to rely on proximate factors to predict conditions nine months in advance. External influences that re-align reproduction to match a phase-shift in environmental conditions have to either adjust the period of the biological clock, or else induce a phase-shift, to bring breeding back into line with prevailing conditions. These are non-genomic factors: they influence the expression of genes, and therefore phenotype, without altering the DNA.

Stages of reproduction relate temporally with the endogenous rhythm, but individual flying-foxes may need to make fine adjustments in their own timing. To do this they probably monitor a suite of environmental conditions. It has been proposed that if a signal changed in isolation from other factors it would be ignored. For example, it is important that a nomadic species be not directly responsive to photoperiod since this differs when they move between latitudes. A reproductive stage would, however, be inhibited if several cues warned against proceeding, e.g. inadequate forage combined with long commuting distances at a time of unfavourable temperatures.

What non-genomic factors regulate flying-fox reproduction? It is likely that rainfall is part of the regulation for the overall rhythm, while current energy balance is probably important for individual animals during many stages of their reproduction.

Key words: chronobiology, reproduction, *Pteropus*, seasonal breeding, circannual reproduction, reproductive physiology, zeitgeber

Genome-encoded endogenous rhythm aligns reproductive timing with average resource availability

An endogenous circannual rhythm sets the timing of seasonal reproduction in flying-foxes (O'Brien 1993). The timing is coded in their genome – i.e. it is in their genes. They are born with the basic framework for seasonal breeding deeply embedded somewhere in their brain.

This means that the rhythm of reproductive events is set internally (endogenous), they do not have to wait for an external cue (such as a solstice, or paperbark flowering), to initiate their breeding. Their internal clock runs with a period of about 12 months; hence it is called circannual – approximately annual. In contrast, the mammalian circadian clock has a period of about 24 hours – approximately daily.

Evolution of an endogenous rhythm aligns particular, important reproductive stages, with resource availability. Often lactation occurs during the best food availability as a result of seasonal breeding; an alternative is that in some mammals the births occur when the temperature is safe for offspring to survive. Having this information programmed into them means that the animals then do not have to predict conditions nine months in advance.

This does not mean that breeding cannot alter its timing. The dates of birth for *Pteropus poliocephalus*, the Grey-

headed Flying-fox, can vary by a few weeks each year (Eby 2008). One of our challenges is to work out why they need to alter, and how they read the environment to calculate the alteration.

Exogenous factors influence gene expression, but not the genes themselves; they fine tune reproduction to the actual prevailing resource availability

External factors that re-align reproduction to match a time-shift in environmental conditions can have one of two effects. Adjusting the period of the biological clock will cause it to run faster or slower. Alternatively, the external influences need to induce a phase-shift, rather like moving the clock forward or back a little, to bring breeding back into line with prevailing conditions.

Extrinsic factors that induce these time changes are non-genomic factors. They are not genetic; they do not alter the DNA. They influence the *expression* of the genes, but they themselves are not genetic – for example they may

function by switching genes on or off, without altering the biological information coded in those genes. The outcomes of the expression of an animal's genes are its phenotype. Any capacity for variation in the phenotype that can be achieved by altering the way genes are expressed constitutes scope for plasticity in the phenotype.

The present review outlines some of the evidence that indicates that flying-fox reproduction is dependent on an endogenous circannual rhythm, and some of the evidence that the timing of that rhythm is sometimes modified by environmental factors.

Genome sets the endogenous rhythm

Endogenous rhythms are interpreted, or read, by the hypothalamus. The hypothalamus output is communicated to the pituitary gland and thence to the reproductive tract, by way of the hypothalamo-pituitary-gonadal system, or HPG axis. Communication from the hypothalamus to the rest of the system is by way of gonadotropin releasing hormone (GnRH), which is produced in the hypothalamus and secreted into portal blood vessels for transport to the pituitary gland. The pituitary gland then produces follicle stimulating hormone (FSH) and luteinising hormone (LH) to communicate with the ovaries and testes regarding production of gametes and sex hormones. The portal vessels are essentially inaccessible for sampling and hormone analysis, but the pituitary outputs are probably more meaningful; this is because the gonadotropes, the cells in the pituitary that produce gonadotropins, FSH and LH, have already partially decoded the hypothalamic signal.

Figure 1 shows part of the pituitary output in female *P. poliocephalus* during different types of reproductive cycles. It is a graph of the endogenous rhythm. It records the pattern of LH in peripheral plasma, so it is a graph of the

information sent out by the hypothalamus and pituitary gland to the reproductive tract each year. In ovariectomized animals (▲), LH in plasma increased in March, eased back after June, and returned to baseline after October. This graph shows three things.

1. It shows that female flying-foxes have anoestrus – when the reproductive tract is switched off (December to mid March). This is new information. A period of infertility in the year ensures that births cannot occur too far out of the normal seasons, so it provides some protection against unwanted or unseasonal births.
2. It sets the rhythm, by setting the start (mid March) and end (November) of reproduction for the year.
3. It demonstrates the presence of negative feedback. The endogenous pattern is only seen in ovariectomized animals. It had been anticipated that the intact animals would express several pulses of LH during the mating season, since LH drives ovulation. At other times, negative feedback from the ovarian oestradiol and progesterone usually keep LH in balance. Meaningful variations in ovarian oestradiol and progesterone have not been found in systemic plasma of flying-foxes, despite several investigations (Martin *et al.* 1995; Towers and Martin 1995). LH concentrations reported in Fig. 1 remained low throughout the year, in intact animals (breeding (■) and non-breeding (◆) females compared with ovariectomized (▲) females). This contrast in secretory patterns clearly demonstrates the presence of negative feedback from the ovaries to the pituitary. If this is not achieved by oestradiol or progesterone, then it may be necessary to posit the presence of a novel steroid, or a non-steroidal hormone.

A non-steroidal hormone usually regulates ovarian feedback on the other gonadotropin, FSH. FSH is in negative feedback with inhibin. Since inhibin is a peptide and not a steroid, it cannot diffuse across blood vessel walls, from the vein to the artery in the ovarian vascular

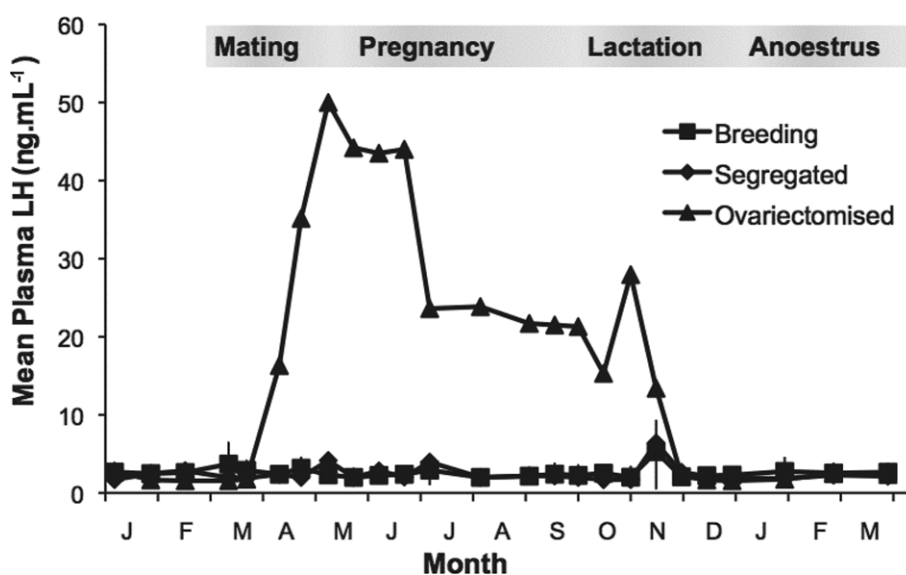


Figure 1. Luteinizing hormone (LH) in female *P. poliocephalus* during three different reproductive cycles: ◆ = segregated; ▲ = ovariectomised; ■ = breeding (O'Brien and Gray 2003). It is one aspect of the endogenous rhythm regulating reproduction in flying-foxes.

complex. Thus it can escape from the ovary into systemic circulation; from there it can influence the pituitary gland. Studies have begun, into FSH in flying-fox plasma (Macdonald *et al.* 2007), to assess the pattern of feedback that regulates it. Comparison between FSH and LH patterns in plasma across the year should elucidate whether LH shows indications of being regulated under similar feedback as FSH. Perhaps in flying-foxes when inhibin suppresses secretion of FSH by the pituitary gland, the secretion of LH is also suppressed.

Alternatively, is there a novel hormone in flying-foxes that is providing negative feedback from ovary to H-P on LH, not known from other species? Is there a discovery just around the corner?

Alternatives do exist for regulation of the LH surge that drives ovulation. In animals that are induced ovulators, nerve signals are sent from the reproductive tract to the hypothalamus as a result of courtship or mating activities. In such species, the neural inputs can trigger the LH surge that precipitates ovulation to occur in response to mating (Milligan 1982). Ovulation in response to mating will only occur if the ovarian follicles have matured in the appropriate hormonal environment during the preceding weeks (Caillol *et al.*, 1986); it is the regulation of LH during these weeks that is currently perplexing.

Hypothalamus

Upstream from the pituitary gland is the hypothalamus. In mammals the hypothalamus has the important function of ensuring that information about the environment is communicated to the various physiological systems that interact with, and adapt their functions to align with, the environment. Being able to graph the endogenous rhythm gives us a tool to monitor responses of the reproductive system to changes in the environment.

Is there evidence for an endogenous rhythm (in contrast to an exogenous cycling factor)?

Yes, see Fig. 1.

Is there evidence that any endogenous rhythm does not act alone, i.e. is there any variation between years?

Yes, see Fig. 2.

Exogenous factors then alter the timing

Over a ten year period (1987 to 1996) births of *P. poliocephalus* in captivity began from 18 Sep to 22 Oct (Fig. 2), at the University of Queensland in Brisbane. This is a difference of 5 weeks. The small population (average 16.8 births/year, range 11–20) remained synchronized with approximately 70% of births occurring within 23 days of the onset of birthing. Similar observations have been made on free range populations in the wild (Eby (2008) found up to 3 weeks variation over a 5 year period at a camp in Sydney, NSW). Consistent seasonality despite the absence of apparent seasonal cues was documented by Baker and Baker (1936), reporting on *P. tonganus geddiei* in the tropical habitat of Vanuatu. Their studies did not attempt to assess the timing over multiple seasons. Examination of broader taxa indicates that slight variations in the timing of births are common. A small number of Pteropodid species use a period of embryonic delay that would alter the duration of pregnancy (Heideman 1989). In the other animals, the variation in birth times directly reflects variation in conception times, and hence in times when ovulation occurred.

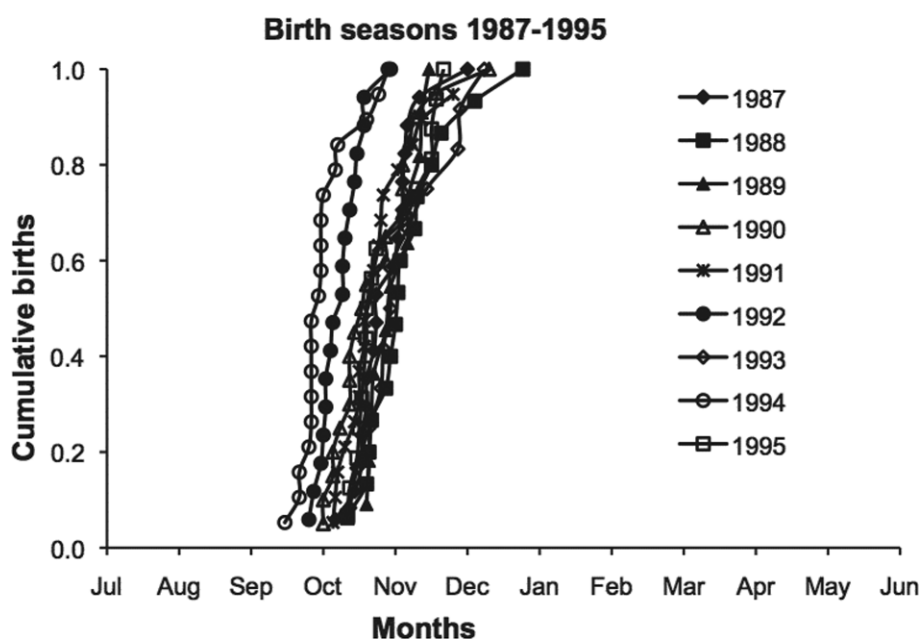


Figure 2. *Pteropus poliocephalus* births in captivity in Queensland remained synchronised each year, but the birthing season moved each year.

Variation in times of ovulation between years implies that resource availability varies temporally between years. Monitoring the environment permits a whole population to predict the need to adjust reproductive timing.

We are faced with

- Why do they need to? and
- How do they do it?

Photoperiod is not an important environmental cue for flying-foxes

Many long-lived mammals from the temperate zone monitor changes in daylength (photoperiod), to tell when it is autumn (Bronson and Heideman 1994). They have become genetically programmed to conceive in autumn so that young will be weaned on high quality fresh forage available in spring.

Flying-foxes ignore photoperiod

O'Brien *et al.* (1993) housed male *P. scapulatus* in artificial daylength conditions that were designed to mimic living at different latitudes. The animals ignored the photoperiod signals, and continued to display the annual patterns of fertility increase and decrease that were seen in control animals that had been housed outdoors under normal daylight in Brisbane. In a follow-up study, animals were housed under conditions of unchanging daylength (12 h of light: 12 h of dark) for four years. Throughout that time there was no indication that they needed photoperiod to signal the time of year – their testis size increased and decreased regularly as expected (Fig. 3) despite the removal of photoperiod cues altogether.

An alternative approach is to examine birth times from different latitudes. In nature, differences in daylength occur with changes in latitude. In late summer and autumn, daylength around the equator remains close to

12 hours, whereas daylength in higher latitudes changes rapidly from long days (e.g. 16h daylight at the summer solstice) to short days (e.g. 8h daylight at the winter solstice). If conception times begin in synchrony with predictable short daylength, then births could occur earlier near the equator, and later at higher latitudes which do not reach short days until after the autumn equinox (Bronson and Heideman 1994).

To test the effect of latitude on reproduction in flying-foxes, dates of birth of *P. alecto* were pooled over a ten year period (Martin 1997). The dates include all the variation, that occurs from one year to the next, which was seen in Fig. 2, giving rise to some noise in the data (Fig. 4). Despite this, the data in all the plots overlap, illustrating that average times of birth were the same whether the *P. alecto* populations were in Townsville, Brisbane or northern NSW, and whether they were in a captive colony or free range in the wild. Clearly, photoperiod was not providing a strong signal to influence the timing of reproduction in *Pteropus* spp.

Populations of *P. alecto* have also been examined in the Northern Territory of Australia, at latitudes similar to Townsville but at different longitudes (Vardon and Tidemann 1998). Those results will be discussed below.

Bronson model: multiple metabolic and neural pathways with overlap and redundancy

Numerous studies of photoperiod and associated environmental parameters failed to identify the controlling features of reproduction in tropical bats (Heideman and Bronson 1994, Martin *et al.* 1995). Bronson (1998) subsequently pointed out that real animals live in the real world. In the real world, resources are constantly changing, in space and in time. In contrast, to test whether an environmental factor is acting as a cue to animals, the standard scientific approach is to present the animals with variations in that factor, while holding all other factors constant. Bronson pointed out that this presents the animals with the test stimulus in a context that would never occur in its real world. As such, it may be interpreted by the hypothalamus as meaningless; it was

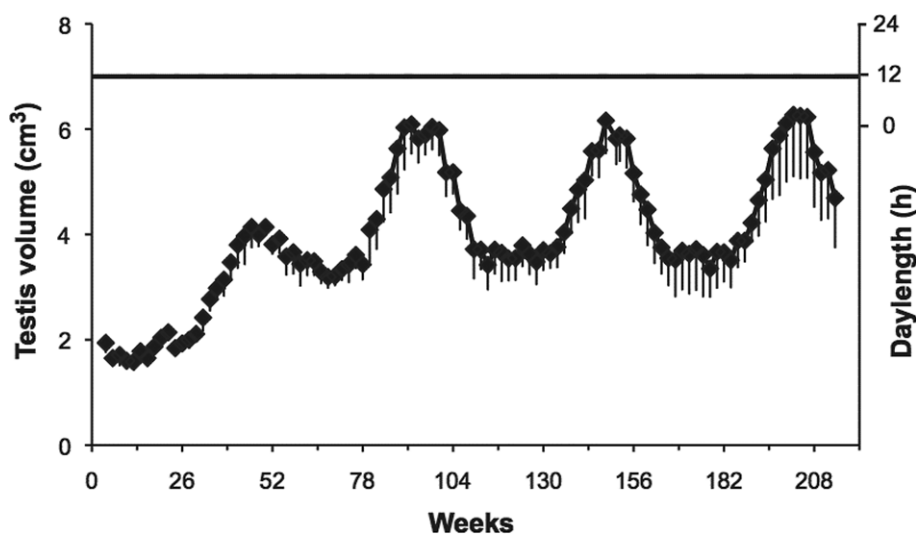


Figure 3. Testis size of *P. scapulatus* followed a remarkably similar seasonal pattern during four years in unchanging daylength (O'Brien, Curlewis and Martin, unpublished data).

not part of the normal fabric of life – so it was ignored. If this is the case, then standard scientific tests will never help us to elucidate the environmental cue regulating reproduction in flying-foxes. Instead, a different theoretic construct is needed. The theory proposed by Bronson was one that attempted to resolve actual control mechanisms that regulate reproduction in natural habitats.

In his review of the relationship between energy balance and ovulation Bronson (1998) concluded that "...females ovulate whenever extant energetic conditions permit unless the process is blocked by non-metabolic stress, social cues or a predictive seasonal cue such as photoperiod" (Bronson 1998). Ergo, follow the endogenous rhythm, encoded in the genome, unless environmental conditions indicate a need to pause. "...most mammals do not use a predictive cue; they reproduce opportunistically in relation to seasonal variation in the energetic characteristics of their environment" (Bronson 1998). The flying-fox appears to be such a mammal that does not use a simple predictive cue, and yet does appear to vary the time-course of breeding in relation to the environment (Fig. 2); environmental conditions do influence the phenotypic expression of the breeding blue-print in the bats' genetic code. Physiologically, "...the complexity of the energetic challenges mammals face in the wild suggests that there are probably multiple metabolic and neural pathways coupling ovulation to energy balance and that these pathways are probably characterized by considerable overlap and redundancy" (Bronson 1998). Evidence suggests that pteropodid reproduction is not blocked by low temperatures (e.g. snow in Bendemeer, NSW, 2001, unpublished data), or long foraging distances etc., but rather by the coincidence of several energetic challenges, such as atypically cold or wet weather occurring during a time of poor quality forage requiring additional energy to be expended on obtaining nutrition.

Perhaps long-lived tropical mammals monitor a dozen cues; whilst ever these cues make sense, the mammals continue to rely on the endogenous rhythm. If a factor indicates that it is not propitious or safe to breed, they ignore it. But, if *many* factors indicate danger, then they should "put reproduction on hold" (Bronson 1998).

The Bronson model applied to flying-fox reproduction

Numerous environmental factors are monitored, and a matrix of information is resolved by the hypothalamus

Stages of reproduction – folliculogenesis, testicular regression, etc. – relate temporally with the endogenous rhythm, but individual flying-foxes may need to make fine adjustments. To do this they probably monitor a suite of environmental conditions. It has been proposed that if a signal changed in isolation from other factors it would be ignored. For example, it is important that a nomadic species be not directly responsive to photoperiod since this differs when they move between latitudes. *Pteropus poliocephalus* has been tracked regularly moving between latitudes in both directions e.g. Eby 1991; Spencer *et al.* 1991; Tidemann and Nelson 2004. A reproductive stage would, however, be inhibited if several cues warned against proceeding, e.g. inadequate forage combined with long commuting distances at a time of unfavourable temperatures. Using numerous cues, with some being ignored at any one time, was referred to as redundancy in the control pathways by Bronson (1998).

The Bronson model changes the task involved in developing an understanding of how the seasonal reproduction of flying-foxes is regulated. We are now not seeking to identify "the" particular environmental cue – rather we are looking for a suite of factors that could alter the gene expression in a given year. The altered gene expression would produce a modified phenotype, one in

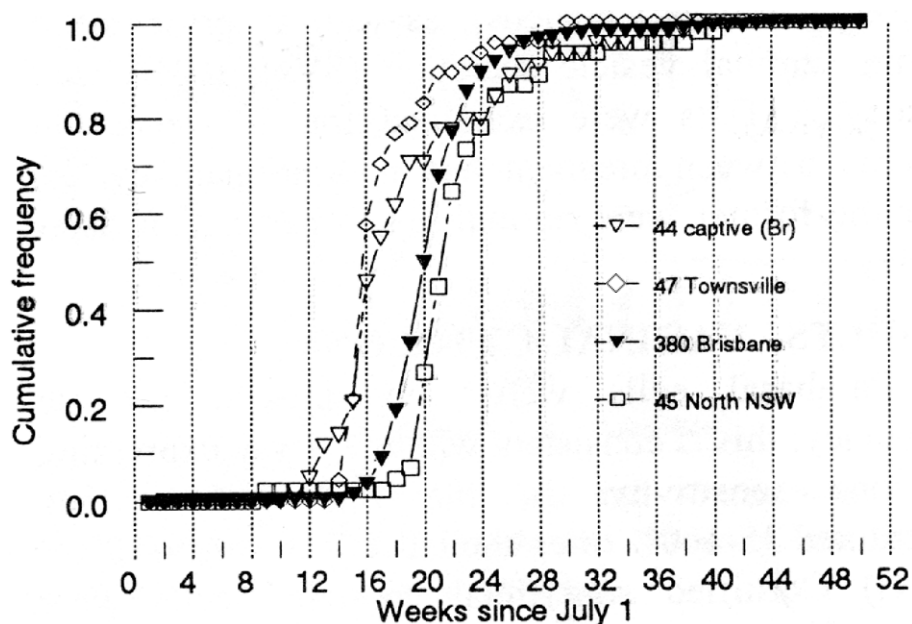


Figure 4. Frequency distribution of births of *P. alecto* at different latitudes (Martin 1997). 44 *P. alecto* births in captivity ∇ = in Brisbane (latitude $-27^{\circ}28'$) are compared with births of 47 free-living *P. alecto* \diamond = in Townsville (lat. $-19^{\circ}15'$), \blacktriangledown = 380 in Brisbane, and \square = 45 in northern NSW (lat. $-30^{\circ}18'$).

which reproduction occurred at a time that suited the environmental conditions occurring during the current breeding cycle. This is phenotypic plasticity.

Possible environmental cues – i.e. what would the flying-foxes be monitoring?

- Photoperiod

Photoperiod is always the first assumption when searching for a zeitgeber. Zeitgebers are cues in the environment that can be used to align physiology with the environment. Daylength is the main signal used by many intensively studied species from the temperate zones, both animal and plant. Variations on this include luminance, which changes during the day, even on the equator, and moonlight, which is relevant to nocturnal hunting and may be used by the hypothalamus for monitoring environmental conditions. As shown above (Fig. 3 and Fig. 4) reproduction of flying-foxes is not controlled by changes in daylength. A possible role for photoperiod to act as one of a suite of factors has not been ruled out. Application of artificial daylengths to male *P. poliocephalus* has identified what appears to be some slight interference with the endogenous rhythm (O'Brien *et al.* 1991; McGuckin and Blackshaw 1992); despite interfering, photoperiod was not able to entrain the physiology to match the applied environmental factor of daylength. Similar results occurred when an equatorial microchiropteran bat, *Anoura geoffroyi* was tested in Trinidad; Heideman and Bronson were able to disrupt the reproductive timing of this bat, but could not entrain it to a new rhythm, despite the use of photoperiod with and without modified temperature (Heideman and Bronson 1994), food quality, light quality (e.g. polarized) and simulated rainfall (Heideman 2000).

- Temperature

Reproduction is energy-dependent. The energy balance for flying-foxes is affected by temperature in a number of ways, most obviously in relation to thermoregulation. Abnormally high temperatures lead to expenditure of energy in cooling and surviving heat waves (Welbergen *et al.* 2008). Large numbers of young have been lost when cold nights occurred at unseasonal times (O'Brien 2007). In contrast, the distribution and quality of forage has led to groups of *P. poliocephalus* spending winter at altitudes of approximately 810m instead of the usual 20m (Eby *et al.* 1999), through winter frosts and even some snow at times (Bendemeer, NSW, 2001, unpublished data). The energy equation includes parameters for calorie intake, temperature, distances travelled for daily foraging, etc. While extremes of temperature could adversely affect the reproductive potential of individuals, there is no evidence that it would be a practical cue for reproductive rhythms of entire species of endothermic mammals, except those that utilise hibernation or torpor, which flying-foxes do not.

- Dietary factors

Many components of diet can influence reproduction. Calcium probably provides an overall constraint on the reproductive output of bats (Barclay 1995; Racey and Entwistle 2000), but is not likely to modify the time-course each season. Attempts to identify dietary cues have included consideration of nutrient levels, especially

nitrogen balance; calories; relevant trace elements; specific plants; and specific plant products such as phytoestrogens. Most of these have not been tested directly in laboratory conditions. Circumstantial evidence is available from long-term studies of reproduction in colonies housed at the University of Queensland. Diets for these animals were kept relatively constant in quality and mix throughout each year; during that time, birthing seasons maintained parity with free-living populations in the same districts, feeding on natural diets (Fig. 4). Thus, diet is not a strong contender as an environmental regulator of pteropodid reproduction.

When reviewing environmental regulation of reproduction in bats, Heideman (2000) implicated some aspect of diet as the ultimate zeitgeber for timing reproduction, that is, one that acts via the genome. Proximate cues could not be identified, and possibilities seemed to be limited by the requirement that any environmental cue would need to be transduced for communication to the hypothalamus or to functions regulated by the hypothalamic–pituitary axis. Progress can now be made, since known physiological mechanisms could provide the necessary characteristics to allow aspects of diet to act as proximate cues. Variations in the quality or quantity of nutrients can be continually monitored to provide information about the immediate environment. There are hormonal responses to changes in calcium, including calcitonin and parathyroid hormone. Calorie balance is managed by a combination of insulin, glucagon, and leptin amongst others. Nitrogen balance influences growth hormone secretion. These examples indicate that neuroendocrine mechanisms do exist that can communicate environmental conditions to the hypothalamus.

- Rainfall

As with other features of the environment, rainfall can be assessed in many different forms. Features that might influence animals include total precipitation, drought, humidity, barometric pressure, rainfall arriving early or late; pattern of rainfall being seasonal (monsoonal, winter dominant, etc) or evenly distributed through the year.

There is some evidence that rainfall may influence the timing of flying-fox reproduction. This evidence comes from two different species of flying-fox, from different regions of the Australian continent, and from different researchers, but the conclusions are similar.

Vardon and Tidemann (1998) concluded that predictability of plant productivity explained reproductive differences between the birthing times of *P. alecto* in the Darwin region of the Northern Territory of Australia (Jan – Mar) compared with those in Townsville and other east coast centres south to Brisbane (Oct – Nov). Their analysis suggested that the key difference was between a strongly seasonal rainfall and a non-seasonal pattern. These patterns of rainfall in different locations, combined with the different temperatures, humidities etc, produced different times of year for maximum plant productivity. At the sites where these analyses were undertaken, *times of flying-fox parturition differed, but was aligned with the local time of maximum plant productivity*. Thus plant productivity appeared to be the ultimate cue with rainfall acting as the local, proximate cue.

In an unrelated, long-term study of birthing of *P. poliocephalus* through its range in eastern Australia, records have demonstrated synchrony in births across seven degrees of latitude (Eby and Jones, unpublished data). Between years there has usually been only slight variation, similar to that found in captive populations (Fig. 2). Most, but not all, of this species' geographic range has high summer rainfall. Eastern parts of Australia have experienced significant droughts in recent years. In one of these years births in different parts of the *P. poliocephalus* distribution were uncoupled, and differed by a month.

Environmental conditions were examined retrospectively. It was found that the late births had occurred in a region where the summer rain was delayed by a month. Populations in the other part of the species' range gave birth at the usual time, and their rainfall pattern had been normal that year (Eby and Jones, pers. comm, 2006). If delaying precipitation by a month would cause a one month delay in the time of maximum plant productivity, then the unusual times of births in *P. poliocephalus* in that year provide evidence for rainfall being a proximate cue for the regulation of seasonal reproduction of Australian flying-foxes.

Corroboration from other species and other countries

There is evidence that some pteropodids in other genera have similar regulatory systems. Correlations between rainfall and birth times for *Cynopterus brachyotis*, *Haplonycteris fischeri* and *Ptenochirus jagorii*, of the Philippines, were examined by Heideman (1995). Although the correlations were statistically significant, there appeared to be additional regulators on reproductive timing involved as well (Heideman 1995).

Environmental factors combine to predict plant productivity

These temporal changes (outlined above) did not occur at the time of birth – conception times (and therefore ovulations) must have varied between years. This means that conditions that will prevail during early lactation e.g. Nov–Dec, were being predicted when folliculogenesis was underway, in Jan–Feb. If numerous factors of those discussed above are negative, then plant productivity cannot be relied upon for the following nine months of reproductive stages. Ovulation and conception should be postponed, or else early pregnancies may be reabsorbed; later pregnancies might be aborted. There have been several recorded instances where poor conditions have been associated with large losses of pregnancies, resulting in reduced numbers of offspring being produced that year (Eby 2000).

As noted above, Vardon and Tidemann (1998) interpreted the birth times of *P. alecto* in Australia as being influenced by the timing of maximum plant productivity. They also identified a number of other species of Pteropodidae, including examples from Africa, Brunei, India and the Philippines, that exhibited birth times that were variously related to this forage availability or its promoter, rainfall, or to maximum production of particular dietary components.

Phenotypic plasticity of mechanisms that regulate reproduction in flying-foxes can now be said to involve many small components rather than a single, easily identifiable pattern. However, in the Australian and African contexts, variations in rainfall may be a useful guide. We must keep in mind the complexity though, since salinity, global warming, and the greenhouse effect will alter the relationships between rainfall, plant productivity, and the other environmental factors that are probably contributing to the regulation of reproduction of flying-foxes. It is to be hoped that the redundancy in the matrix system of cues will allow the animals sufficient flexibility in their reproductive physiology to survive the new environment that is emerging.

• Social environment

Olfactory cues, pheromones, auditory influences, population densities and mix are factors that have not been examined in detail in relation to the reproduction of Australian flying-foxes. There have long been suggestions of important roles for these, and their influences probably confound the effects of other factors being tested, such as photoperiod or diet.

Despite numerous ethological studies over several decades which have examined the reproduction of pteropodid bats, including those from Puddicombe (1981), Markus (2002), and Connell *et al.* (2006), only a few have included physiological correlates, e.g. Nelson (1965), O'Brien and Nankervis (1994), so they will not be reviewed in detail in the present paper. A single example will be given to indicate some exciting developments that are emerging.

Exciting recent developments

Groups of animals housed in single-sex groups have often been used as control groups when investigating the reproductive endocrinology of flying-foxes. Generally no differences have been found between endocrine profiles of segregated and breeding *P. poliocephalus*: prolactin in males (O'Brien *et al.* 1990), LH in females (O'Brien and Gray 2003), oestradiol, progesterone and androgens, except during pregnancy (Martin and Bernard 2000).

A difference recently emerged when follicle stimulating hormone (FSH) was measured in plasma of females through a complete sequence of reproductive stages. It was found that females that participated in courtship and mating (the 'breeding' group in Figure 1) had higher mean concentrations of FSH in their plasma during the time of folliculogenesis than females that could hear and smell but not touch males (the 'segregated' group in Figure 1) (Macdonald 2007). The ovariectomized animals were included, and differences between groups ($p < 0.01$), differences between reproductive stages ($p < 0.02$), and interaction between group and reproduction ($p < 0.01$) were all statistically significant. This implies that male–female interactions may synchronise and/ or facilitate the time of onset of conceptions in normal populations. Male *P. poliocephalus* reach peak fertility before the females. The endocrine response to courtship and/or copulation may be the node where any differences between male and female timing can be resolved.

Summary

Basic frame is set by an endogenous rhythm (genome);

Monitor environment, and shift timing if enough environmental factors warn against proceeding.

Conclusion: phenotypic plasticity of flying-fox reproduction aligns the genome-encoded rhythm to local environmental conditions

Pteropodid bats have an approximate timeframe for reproduction encoded in their genome, as illustrated in Fig.1, and an approximate starting date for that

timeframe. Environmental cues may switch genes off for a time, leading to specific matching between environment and phenotype. Evidence points to plant productivity being the ultimate zeitgeber that the animals need to fit their reproduction to, in order to maximize their reproductive success. Daily energy balance is probably important for individual animals as a means of transducing information regarding regional rainfall into a form that can be interpreted by the hypothalamus. In this way, a variety of local indicators probably work with rainfall to act as proximate cues that adjust or fine-tune the small physiological steps along the way, leading to parturition and early lactation occurring at the time of maximum plant productivity.

Acknowledgements

Len Martin and his team J.H. Kennedy, M.A. McGuckin, C.S.T. Pow, P.A. Towers – e.g. data assembled in graph of birth seasons; Peggy Eby always inspirational, and whose canny observations have contributed to the thinking presented here; K-A. Gray and J.D. Curlewis, co-authors on some of the work I have presented here; B.R. Boland, M-C.G. Fisher, J.V.

Forest, a group of people who have been studying reproductive behaviour with me, and this has driven much of the thinking process for this topic; and my current team, R.B. Gear, J.A. Hopper, A.A. Macdonald and D.F. Melville, who keep adding new dimensions to the solutions.

References

- Baker, J.R. and Baker, Z. 1936. The seasons in a tropical rain-forest (New Hebrides).—Part 3. Fruit-bats (Pteropidae). *Journal of the Linnaean Society, Zoology* 40: 123–141.
- Barclay, R.M.R. 1995. Does energy or calcium availability constrain reproduction by bats? *Symposium of the Zoological Society, London* 67: 245–258.
- Bronson, F.H. 1998. Energy balance and ovulation: small cages versus natural habitats. *Reproduction, Fertility and Development* 10: 127–137.
- Bronson, F.H. and Heideman, P.D. 1994. Seasonal regulation of reproduction in mammals. Pp. 541–584 in *The Physiology of Reproduction* (2nd ed.), edited by E. Knobil and J.D. Neill. Raven, New York.
- Caillol, M., Meunier, M., Mondain-Monval, M., and Simon, P. 1986. Seasonal variations in the pituitary response to LHRH in the brown hare (*Lepus europaeus*). *Journal of Reproduction and Fertility*, 78: 479–486.
- Connell, K.A., Munro, U. and Torpy, F.R. 2006. Daytime behaviour of the grey-headed flying fox *Pteropus poliocephalus* Temminck (Pteropodidae: Megachiroptera) at an autumn/winter roost. *Australian Mammalogy* 28: 7–14.
- Eby, P. 1991. Seasonal movements of grey-headed flying foxes, *Pteropus poliocephalus* (Chiroptera: Pteropodidae), from two maternity camps in northern New South Wales. *Wildlife Research* 18: 547–559.
- Eby, P. 1995. *The biology and management of flying foxes in NSW*. Species Management Report. Hurstville, NSW: NSW NPWS. 18: 72pp. ISBN 0 7310 0829 4
- Eby, P. 2000. Low reproductive success in grey-headed flying foxes associated with a short period of food scarcity. *Australasian Bat Society Newsletter* No. 14, pp.17–20. ISSN: 1448-5877.
- Eby, P. 2008. Overview of flying-fox biology and ecology. Pp. 3–55 in *Managing Flying-fox Camps for Land Managers: Workshop Proceedings*. Department of Environment and Climate Change (NSW).
- Eby, P., Richards, G., Collins, L. and Parry-Jones, K. 1999. The distribution, abundance and vulnerability to population reduction of a nomadic nectarivore, the grey-headed flying-fox, *Pteropus poliocephalus* in NSW during a period of resource concentration. *Australian Zoologist* 31: 240–253.
- Fox, S., Spencer, H. and O'Brien, G.M. 2008. Analysis of twinning in flying-foxes (Megachiroptera) reveals superfoetation and multiple-paternity. *Acta Chiropterologica* 10: 271–278.
- Heideman, P.D. 1989. Delayed development in Fischer's pygmy fruit bat, *Haplonycteris fischeri*, in the Philippines. *Journal of Reproduction and Fertility* 85: 363–382
- Heideman, P.D. 1995. Synchrony and seasonality of reproduction in tropical bats. *Symposium of the Zoological Society, London* 67: 151–165.
- Heideman, P.D. 2000. Environmental regulation of reproduction. Pp. 469–499 in *Reproductive Biology of Bats*, edited by E.G. Crichton and P.H. Krutzsch. Academic Press, London.
- Heideman, P.D. and Bronson, F.H. 1994. An endogenous circannual rhythm of reproduction in a tropical bat, *Anoura geoffroyi*, is not entrained by photoperiod. *Biology of Reproduction* 50: 607–614.
- Kennedy, J.H. 1992. *Regulation of Ovarian Function in Australian Flying-foxes*. PhD thesis, The University of Queensland, Brisbane.
- Macdonald, A.A. 2007. *Follicle stimulating hormone regulation of reproduction in Australian flying-foxes*. BSc(Hons) Thesis, University of New England, Armidale, NSW.

- Macdonald, A.A., Gray, K.A. and O'Brien, G.M. 2007. Gonadotrophin hormones in flying-fox plasma during key reproductive stages. *Proceedings of the Australian Physiological Society* 38: 87P. <http://www.apps.org.au/Proceedings/38/87P/>
- Marcus, N. 2002. Behaviour of the black flying fox *Pteropus alecto*: 2. Territoriality and courtship. *Acta Chiropterologica* 4: 153–166.
- Martin, L. 1997. What regulates seasonal breeding in Australian flying foxes (genus *Pteropus*)? *Proceedings of the Australian Society for Reproductive Biology* 28: 49.
- Martin, L. and Bernard, R.T.F. 2000. Endocrine regulation of reproduction in bats: the role of circulating gonadal hormones. Pp. 27–64 in *Reproductive Biology of Bats*, edited by E.G. Crichton and P.H. Krutzsch. Academic Press, London.
- Martin, L., Kennedy, J.H., Little, L., Luckhoff, H.C., O'Brien, G.M., Pow, C.S.T., Towers, P.A., Waldon, A.K. and Wang, D.Y. 1995. The reproductive biology of Australian flying-foxes (genus *Pteropus*). *Symposium of the Zoological Society, London* 67: 167–184.
- McGuckin, M.A. and Blackshaw, A.W. 1992. Effects of photoperiod on the reproductive physiology of male flying foxes, *Pteropus poliocephalus*. *Reproduction, Fertility and Development* 4: 43–53.
- McWilliam, A.N. 1985. The feeding ecology of *Pteropus* in north-eastern New South Wales, Australia. *Myotis* 23–24: 201–208.
- Milligan, S. R. 1982. Induced ovulation in mammals. Pp. 1–46 in *Oxford Reviews of Reproductive Biology*, edited by C.A. Finn, volume 4. Oxford University Press, Oxford.
- Nelson, J.E. 1965. Behaviour of Australian Pteropodidae (Megachiroptera). *Animal Behaviour* 13: 544–557.
- O'Brien, G.M. 1993. Seasonal reproduction in flying foxes, reviewed in the context of other tropical mammals. *Reproduction, Fertility and Development* 5: 499–521.
- O'Brien, G.M. 1999. What is the timecourse of courtship in *Pteropus scapulatus*, little red flying foxes? *Proceedings of the Australian and New Zealand Society for Comparative Physiology and Biochemistry* 16: 16. ISBN 1 86389 610 4.
- O'Brien, G.M. 2007. Prevention is better than cure when managing flying-fox camps. *Australasian Bat Society Newsletter* No. 28, pp.47–53 ISSN: 1448-5877.
- O'Brien, G.M., Curlewis, J.D. and Martin, L. 1990. Plasma prolactin levels in male grey-headed flying foxes. *Proceedings of the Australian Physiological and Pharmacological Society* 21: 65P. ISSN 0067-2084.
- O'Brien, G.M., Curlewis, J.D. and Martin, L. 1991. Unusual reproductive photoresponsiveness of male greyheaded flying foxes (*Pteropus poliocephalus*). *Proceedings of the Australian Society for Reproductive Biology* 23: 160.
- O'Brien, G.M., Curlewis, J.D. and Martin, L. 1993. Effect of photoperiod on the annual cycle of testis growth in a tropical mammal, the little red flying fox, *Pteropus scapulatus*. *Journal of Reproduction and Fertility* 98: 121–127.
- O'Brien, G.M. and Gray, K.A. 2003. Central program and ovarian feedback both influence LH secretion in flying-foxes. *Proceedings of the Australian Physiological and Pharmacological Society* 33: 77P. ISSN 0067-2084.
- O'Brien, G.M., McFarlane, J.R. and Kearney, P.J. 1999. New LH assay casts light on the riddle of flying fox reproduction. *Proceedings of the Australian Physiological and Pharmacological Society* 30: 33P. ISSN 0067-2084.
- O'Brien, G.M., McFarlane, J.R. and Kearney, P.J. 2001. Pituitary production of LH in female flying foxes, Genus *Pteropus*. Abstracts for the 32nd Annual Conference of the Society for Reproductive Biology, Gold Coast, Qld, 9–12 September 2001, Abstract 82 ISSN 0812-7662.
- O'Brien, G.M. and Nankervis, R.F. 1994. Coital behavior of male *Pteropus scapulatus* (little red flying foxes) in captivity. *Physiology and Behavior* 56: 471–478.
- Pow, C.S.T. and Martin, L. 1994. The ovarian-uterine vasculature in relation to unilateral endometrial growth in flying foxes (genus *Pteropus*, suborder Megachiroptera, order Chiroptera). *Journal of Reproduction and Fertility* 101: 247–255.
- Puddicombe, R. 1981. *A behavioural study of the grey-headed flying fox Pteropus poliocephalus (Megachiroptera)*. BSc(Hons) Thesis, University of New England, Armidale NSW.
- Racey, P.A. and Entwistle, A.C. 2000. Life-history and reproductive strategies of bats. Pp. 363–414 in *Reproductive Biology of Bats*, edited by E.G. Crichton and P.H. Krutzsch. Academic Press, London.
- Spencer, H.J., Palmer, C. and Parry-Jones, K. 1991. Movements of fruit-bats in eastern Australia, determined by using radio-tracking. *Wildlife Research* 18: 463–468.
- Tidemann, C.R. and Nelson, J.E. 2004. Long-distance movements of the grey-headed flying-fox (*Pteropus poliocephalus*). *Journal of Zoology, London* 263: 141–146.
- Towers, P.A. and Martin, L. 1985. Some aspects of female reproduction in the Grey-headed Flying-fox, *Pteropus poliocephalus* (Megachiroptera: Pteropodidae). *Australian Mammalogy* 8: 257–63.
- Vardon, M.J. and Tidemann, C.R. 1998. Reproduction, growth and maturity in the black flying-fox, *Pteropus alecto* (Megachiroptera: Pteropodidae). *Australian Journal of Zoology* 46: 329–344.
- Welbergen, J.A., Klose, S.M., Markus, N. and Eby, P. 2008. Climate change and the effects of temperature extremes on Australian flying-foxes. *Proceedings. Biological sciences / The Royal Society* 275: 419–425 doi:10.1098/rspb.2007.1385.