

Convergence in reflectance spectra among treefrogs

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Abstract. Infrared reflectance occurs in a wide diversity of vertebrates, but its biological significance remains largely unknown. Biological roles of visual crypticity, thermal crypticity, and reduction in heat load were explored in frogs with quantitative data on reflectance capability. Reflectance spectra between 450 and 1000 nm were measured on two categories of green treefrogs, one of which was thought to reflect near infrared radiation, the other of which was not. In a paired comparison, leaf-sitting frog species from five genera (*Litoria infrafrenata* Gunther, *Agalychnis callidryas* Cope, *Phyllomedusa sauvagii* Boulenger, *Pachymedusa dacnicolor* Cope, and *Rhacophorus malabaricus* Jerdon) were selected on the basis of previous infrared photographic observations. Analyses of reflectance spectra revealed that in all cases, the leaf-sitting, near infrared reflecting frogs had a reflectance spectrum that contrasted significantly from that of the con-familial non near infrared reflecting frog species (*Osteopilus septentrionalis* Dumeril & Bibron, *Hyla cinerea* Schneider, and *Polypedates leucomystax* Gravenhorst). In all cases, there was a strong convergence in the reflectance spectra of these distantly related near infrared reflecting frog species. Additionally, for all five near infrared reflecting frogs, their reflectance spectra was very similar over the 450–1000 nm waveband to that of leaves, their typical resting sites. An energy budget analysis of the reflectance spectra suggests that thermoregulation by itself is not the primary biological role of high near infrared reflectance. Rather, this capacity appears to be most likely associated with predator avoidance, functioning to make frogs visually and, perhaps, thermally cryptic to their predators. The convergence of similar patterns of spectral reflectance between distantly related species within the same family and between unrelated species in different families further suggests that reflectance in the

near infrared wavelengths has adaptive significance.

Key-words: Convergent evolution, Hylidae, infrared reflectance, Rhacophoridae, treefrog

Introduction

Animal colour patterns that mimic backgrounds have often been associated with increased protection from predation (Cott, 1940; Burt, 1979). Over a broad taxonomic range, there are numerous examples of animal reflectance spectra in the visible wavelengths (400–700 nm), which mimic that of the animal's background. Extension of these observations into the near infrared wavebands (700–1200 nm) has been very limited, despite the indication that potential predators may be sensitive over portions of the near infrared waveband (Vanderplank, 1934; Cott, 1940; Krempels, 1988). Using near infrared colour photography, Dodd (1981) reported that some species of chameleons could reflect near infrared radiation while others could not. Also on the basis of near infrared colour photography, several species of neotropical treefrogs have been shown to reflect light in the 700–900 nm waveband of the near infrared region (Schwalm, Starrett & McDiarmid, 1977). Visual crypticity, thermal crypticity and reduction of heat load have all been suggested as possible biological roles for this capacity. No further work has been done to detail reflectance spectra on frogs in an attempt to uncover the exact function played by near infrared reflectance in frogs. In this study, quantitative reflectance spectra were recorded for four species of New World treefrogs (family Hylidae), and one Old World treefrog (family Rhacophoridae) to test further hypotheses of the possible biological roles suggested in earlier work and to verify the independent evolution and degree of convergence among these unrelated species of frogs.

Methods and materials

Five species of leaf-sitting frogs were examined for near infrared reflectance capability: *Rhacophorus malabaricus* Jerdon (Family Rhacophoridae);

Litoria infrafronata Gunther (subfamily Pelodyrininae, Family Hylidae); *Pachymedusa dacnicolor* Cope; *Agalychnis callidryas* Cope; and, *Phyllomedusa sauvagii* Boulenger (subfamily Phyllomedusinae, Family Hylidae). These species were chosen because previous studies using near infrared colour photography had indicated that these frogs had the capacity to reflect near infrared wavelengths (Cott, 1940; Schwalm *et al.*, 1977; S.B. Emerson, unpublished observation). Additionally, three species belonging to the same families but not thought to reflect near infrared wavelengths (Schwalm *et al.*, 1977; P.A. Schwalm, unpublished observation) were used as controls and similarly tested. Those frogs were *Polypedates leucomystax* Gravenhorst (Rhacophoridae), *Hyla cinerea* Schneider (Hylidae) and *Osteopilus septentrionalis* Dumeril & Bibron (Hylidae).

Reflectance is a measure of the fraction of the incident radiation at a specific wavelength that is neither absorbed nor transmitted by the surface. Reflectance spectra were obtained using a spectral radiometer with an attached 8 cm diameter Taylor integrating sphere (model LI-1800, LiCor Instr., Lincoln, Nebraska, USA). The dorsum of the animal was placed over the 1.5 cm diameter sample opening of the integrating sphere. Three spectra at 20 nm intervals were measured on each individual frog. Over the short time it took to take three measurements at each wavelength (<3 s), there was no detectable shift in reflectance. Longer-term variability was examined by repeatedly measuring the same individual frog under similar conditions on 3 separate days. In this case the average reflectance values at any specific wavelength had 95% confidence intervals of $\pm 1.0\%$ or less. Individual variation was determined by measuring the reflectance spectra of four individuals of *R. malabaricus*. Again, little variation was observed among individuals in their reflectance spectra. The average reflectance values at any specific wavelength always had a 95% confidence interval less than $\pm 1.2\%$.

The potential thermoregulatory advantage of reflecting or absorbing near infrared wavelengths was examined by using standard energy budget equations (Gates, 1980) in which all parameters except for the per cent near infrared reflectance were held constant. We used reflectance spectra of *R. malabaricus* and *P. leucomystax* for this exercise. Some of the other near infrared reflecting frogs we tested have been shown to have specialized morphology and behaviour for reducing evaporative water loss (Blaylock, Ruibal & Platt-

Aloia, 1976; Bentley & Yorrio, 1979; Withers, Hillman & Drewes, 1984). We were interested in examining the effect of differential reflectance on body temperature independent from those differences in the rate of evaporative water loss. *R. malabaricus* lacks the wiping behaviour and special lipid secreting skin glands that characterize the near infrared reflecting frogs with lower levels of evaporative water loss (S.B. Emerson, unpublished observation), and we therefore assume that it has an evaporative water loss similar to the closely related but non near infrared reflecting *P. leucomystax*.

For model calculations, conditions were chosen that would maximize the effects of the observed near infrared reflectivity. Animal temperatures were calculated using the following environmental parameters: total incident solar radiation (1000 W m^{-2}); air, leaf and soil temperatures of 22°C ; a relative humidity of 90%; a barometric pressure of 1000 mb; and, a wind speed of 0.5 m s^{-1} . Frogs had a width of 2.5 cm, a surface conductance to water loss of $0.1 \text{ mmol m}^{-2} \text{ s}^{-1}$, and a visible absorptance to solar radiation (400–700 nm) of 80%. Absorptance to near infrared radiation (700–3000 nm) was set at 50% for the near infrared reflecting frog and 60% for the non near infrared reflecting frog. Animal surface conductances to water loss were determined from the evaporation rate in the literature for *P. leucomystax* (Shoemaker & McClanahan, 1980).

Thermal crypticity by frogs in their proximal biological environment was evaluated by monitoring the surface temperatures of *R. malabaricus* on leaves of *Anona spraguei* under varying light levels in a greenhouse. Initial greenhouse conditions included an air temperature of 25°C , a relative humidity of 60%, and wind speeds less than 0.5 m s^{-1} . Leaf temperature was 22°C . Frog and leaf surface temperatures were measured at 2 min intervals over 40 min periods with an infrared thermometer from a distance of 50 cm (Instatherm model 14-220D, Barnes Engineering, Stamford, Connecticut, USA).

Results

While all the frogs analysed could be visually classified as green, their reflectance spectra were different. The near infrared reflecting frogs (*A. callidryas*, *L. infrafronata*, *P. dacnicolor*, *P. sauvagii* and *R. malabaricus*) in each case exhibited a low reflectance over the visible wavelengths (400–700 nm) with a small increase at the green wavelengths, but then a pronounced increase in reflectance

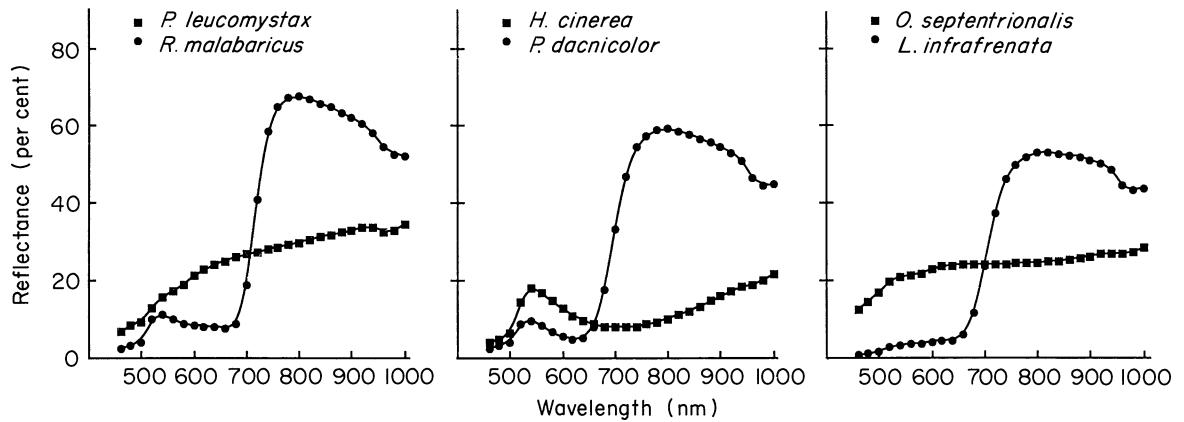


Fig. 1. Reflectance spectra of three unrelated leaf-sitting frogs (●) compared to those of confamilial control species (■). Each data point represents the mean of three readings.

tance over the near infrared wavelengths (700–1000 nm) (Figs. 1 and 2). In contrast, the non near infrared reflecting frogs (*H. cinerea*, *O. septentrionalis* and *P. leucomystax*) had overall greater reflectances in the visible wavelengths, but did not exhibit a pronounced increase in reflectance at the visible near infrared boundary. In all cases, the non near infrared reflecting frogs had lower reflectances in the 700–1000 nm waveband. The flatness of the reflectance response in the non near infrared reflecting frogs contrasted greatly to that of the near infrared reflecting frogs. In all cases, there was greater similarity in the reflectance spectra of distantly or unrelated near infrared reflecting frog species than there was in the reflectance spectra of those frogs and confamilial non near infrared reflecting frogs (Fig. 1). As a corollary, the reflectance spectra of the non near infrared reflecting frogs were also remarkably similar despite the lack of a close phylogenetic relationship. While the control frogs do not reflect as high a percentage of the infrared wavelengths as the leaf-sitting frogs, the data show that these frogs, originally thought to be non-reflecting from infrared photography, did reflect up to 30% of near infrared wavelengths.

The reflectance spectra of *A. callidryas* and *P. sauvagii* were compared to the reflectance spectrum of the leaf of *Anona spraguei* (Fig. 2). This plant is commonly used by *A. callidryas* as a diurnal resting site (P.D. Coley, personal communication). There was a striking similarity in the reflectance patterns of the frogs and leaf. All three spectra exhibited a reflectance peak between 500 and 600 nm (green wavelengths) and a dramatic large increase in reflectance in the near infrared wavelengths (700–1000 nm).

Nearly half of the incident solar energy is in the near infrared range (Gates, 1980). Therefore increased reflectance of these wavelengths could provide a significant thermoregulatory advantage and reduce evaporative water loss rates. Using the incident solar radiation spectrum for an optical air mass of 1.0, the calculated near infrared reflectances of *R. malabaricus* (near infrared reflecting) and *P. leucomystax* (control frog) were 27.4% and 19.3%, respectively. Inserting these absorptance differences into surface energy budget equations predicts that these differences in reflectance would produce less than a 2°C difference in temperature. More significant differences in body surface temperature did not arise because while *R.*

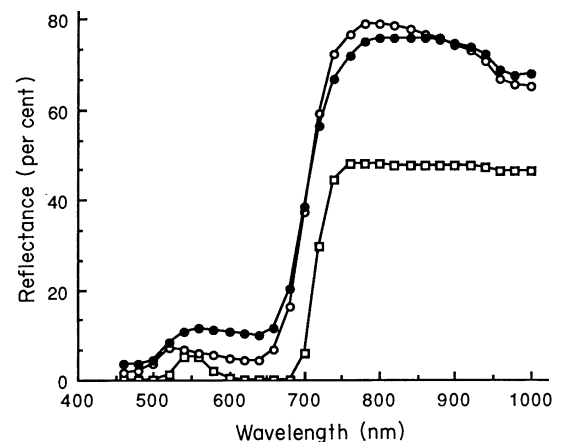


Fig. 2. Reflectance spectra of the neotropical leaf-sitting frogs *Agalychnis callidryas* (○) and *Phyllomedusa sauvagii* (●) compared to that of the leaf of the tropical tree *Anona spraguei* (□). *Anona* leaves are known to be resting sites for *Agalychnis callidryas*.

malabaricus reflected a greater fraction of near infrared wavelengths, *P. leucomystax* reflected a greater fraction of the solar energy in the visible light wavelengths.

Such small differences in body surface temperatures between both types of frogs could be expected to provide thermal crypticity if the frog surface temperatures do not differ from that of the leaves upon which they rest diurnally. When both frog surface and leaf temperatures were monitored in the greenhouse, the temperatures did not differ on average by more than 0.35°C, despite a 5°C range in absolute temperature because of changing irradiance levels.

Discussion

Convergence often results when similar selective pressures work on unrelated or distantly related organisms that are morphologically dissimilar. Immunological and morphological studies on these frogs have demonstrated that the phyllo-medusine and pelodyrine hylids are not closely related (Maxson, 1976; Tyler & Davies; 1978), indicating that there are likely to have been independent origins for increased near infrared reflectance within the family Hylidae. The presence of increased near infrared reflectance in an unrelated member of the Rhacophoridae suggests a third independent origin of this capacity. Through near infrared colour photography, species of another New World treefrog family, the Centrolenidae, have also been shown to reflect near infrared light, representing a fourth potential case of convergence (Schwalm *et al.*, 1977). (The Centrolenidae are generally thought to be derived from the Hylidae [Duellman & Trueb, 1986], but the precise relationships are unknown. Near infrared reflectance could be a shared derived character between the centrolenids and their hylid sister group. In that case its presence in the centrolenids would not be an independent convergence.)

Interestingly the near infrared reflecting characteristics of Old and New World leaf-sitting frogs do not appear to result from an identical organization and structure of skin chromatophores. The hylid and centrolenid frogs differ from each other in the morphology of the chromatophores, and they both vary from the pattern seen in the skin of *R. malabaricus* (Schwalm & McNulty, 1980; S.B. Emerson, unpublished observation).

The quantification of reflectance capability provides some insight into the probable biological role of near infrared reflectance in leaf-sitting frogs. The small difference in the overall reflec-

tance between control and near infrared reflecting frogs and the resulting small calculated differences in surface temperatures suggest that regulation of heat load is not the primary biological role of the increased near infrared reflectance capability. Rather, crypticity emerges as a more likely explanation for this capacity.

Snake infrared receptors detect thermal differences from a background field which is defined by the average radiation from all objects in the receptive field (Bullock & Cowles, 1952). The close similarity of temperature between leaf and leaf-sitting frog suggests that the frog could easily blend in as part of the background thermal environment of the leaf surface. The frogs also share a behavioural similarity consistent with a crypticity hypothesis. Unlike most other anurans, leaf sitters are slow moving and relatively difficult to disturb (Pyburn, 1970, 1980; Cannatella, 1980; S.B. Emerson, personal observation). They usually remain sedentary and motionless when initially approached by an intruder.

While snake infrared receptors are extremely sensitive to intermediate and long wavelengths of infrared radiation, they do have a very low sensitivity to wavelengths shorter than 1000nm (Bullock & Cowles, 1952). The ability of leaf-sitting frogs to reflect these near infrared wavelengths is therefore cryptic only in the sense that it contributes to the overall body temperature of the animal. Our energy budget analyses suggest that there will be little temperature difference between near infrared reflecting and non near infrared reflecting frogs. Furthermore, Tracy (1979) has calculated that frog absorptance differences of as much as 20% will have negligible effects on both temperature and water loss rates because of the normally high rates of evaporation from the frog's surface. However, if predators such as snakes and birds could see in the near infrared range, infrared reflectance could result in the frog being visually cryptic as well. Recent work indicates that such near infrared sensitivity occurs in at least some potential frog predators (Krempels, 1988), implying that a strong, precise matching of reflectance spectra by the frog to that of the leaf may be essential for visual crypticity.

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