In *Vivo* Skin Reflectance of the Wall Lizard, *Podarcis muralis*

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A portable optical-fiber spectrum analyzer operating in the visible and near-infrared range was used to measure *in vivo* the skin reflectance of lizards of the genus *Podarcis*. The investigations, which we performed in connection with a study of the biological problem of the microinsular melanism, are quite safe for the examined animals and can be easily extended to spectroscopic and/or energy input studies in other animals.

Index Headings: Optical fiber; Reflectance spectroscopy; NIR reflectance; *In vivo* skin reflectance.

INTRODUCTION

The external colors of animals may have manifold functions: defensive, sexual, camouflagic (either to hide the animal or to allow an easier approach to the prey), and so on. Accordingly, a huge number of studies on the physics and chemistry of animal colors in relation to ecology, taxonomy, physiology, etc., have been reported. Apart from the old but still valid works by Mason,[1] we recommend Refs. 2 and 3 (and papers quoted therein) to give a panoramic view of the problem to the interested reader.

From a biological standpoint, our main focus has been related to the problem of the microinsular melanism. Here, we intend to present only a suggestion of the biological implications of our work (further details will be given in a successive paper, now in preparation), and we will focus primarily on the spectroscopic aspect of our investigation. Indeed, at present optical-fiber technology allows reflectance spectra to be recorded up to the near-infrared (NIR) region quickly and without any stress for the animal. The extension into the NIR range was important for us, because we are interested in being able to eventually study the thermo-regulatory role of the melanism.

Some lizard populations of the genus *Podarcis* that inhabit the Mediterranean rocky islets often show a different degree of skin darkening (melanism and/or cyanism), which occurs too frequently to be regarded as being devoid of any adaptative value. Granting that dark skin should increase the heating rate in such heliothermic animals as *Podarcis*, several authors[4–6] have stressed the thermo-regulatory function of melanism in a microinsular environment. Some of our previous studies[7] have demonstrated that a slight difference exists in the skin reflectance between melanic and "light" *P. muralis* (for definition, see below) in the 0.38–0.80 μm range, and the heating rate, measured in immobilized specimens, did not differ between melanic and "light" lizards.[8] Since these studies have been carried out with analysis in the visible range only, we decided to carry out a new series of experiments measuring the skin reflectance in the visible and NIR range. In order to estimate the energy input in the same interval, spectra were then normalized, under the assumption that the sun at sea level was the source of energy.

MATERIALS AND METHODS

Podarcis muralis is a small- to medium-sized European lacertid, whose weight does not usually exceed 10 g, showing a remarkable chromatic polymorphism and a number of more or less melanic microinsular populations (or subspecies).

We tested adult male Wall lizards belonging to the following subspecies: *Podarcis muralis brueggemanni* [three specimens from Florence, Italy, with a light-brown to green dorsal ground coloration and a fine black or dark-brown reticulation ("light" animals)]; and *P. muralis marcuccii* [three specimens from Argentarola Islet (Monte Argentario, Grosseto, Italy), with dark-brown to blackish dorsal ground coloration and a rough black reticulation ("dark" animals)].

The diffuse reflectance was measured, in live lizards, with the use of a spectrum analyzer (Guided Wave, Model 260) equipped with 316SS optical fibers, operating in the range 0.40–2.20 μm. The optical-fiber bundle is constituted of nine sending and ten receiving fibers. We made a necessary modification in a wand-type probe by attaching onto the tip of the probe a drilled cylinder (ϕ = 12 mm) of blackened PVC, supplied with an O-ring, in order to exclude external light and to collect the maximum of back-scattered light (our device kept the tip of the probe about 3 mm apart from the lizard skin). The whole probe was then fastened to the animal with an adhesive tape. Through the optical fiber, the radiation from a 20-W tungsten-halogen lamp is directed at the animal. The irradiated surface is 0.3 cm^2, and the mean irradiance measured with a radiometer (Photodyne, Model 350) over the above spot, for radiation in the range 0.4–1.15 μm, is 30 mW/cm^2.

It is important to recall that, in contrast to the external integrating sphere used in our previous paper,[7] the present method does not allow collection of all the back-scattered radiation because of the partial coupling to the
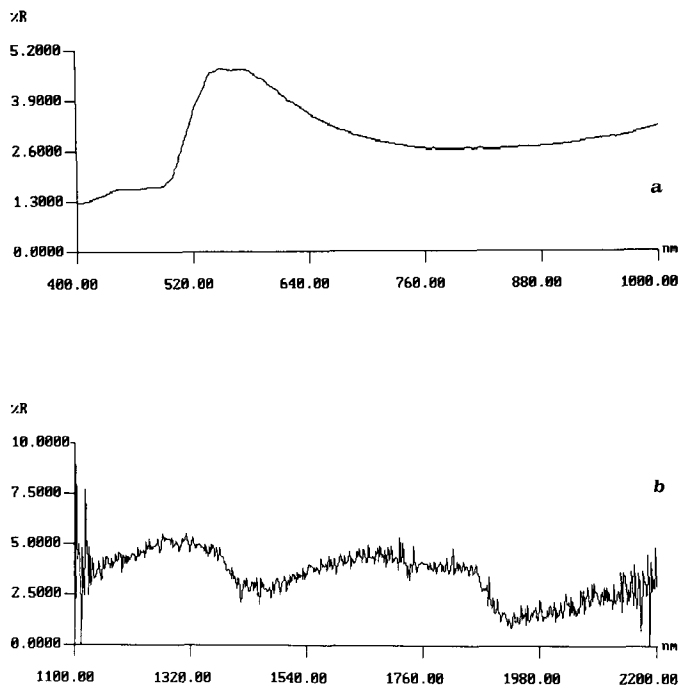
APPLIED SPECTROSCOPY

FIG. 1. Skin reflectance of a specimen of *P. muralis brueggemanni*; (a) range 0.4–1.0 μm, spectrum recorded with a silicon detector and an 800 L/mm grating; (b) range 1.1–2.2 μm, spectrum recorded with a PbS detector and a 300 L/mm grating.
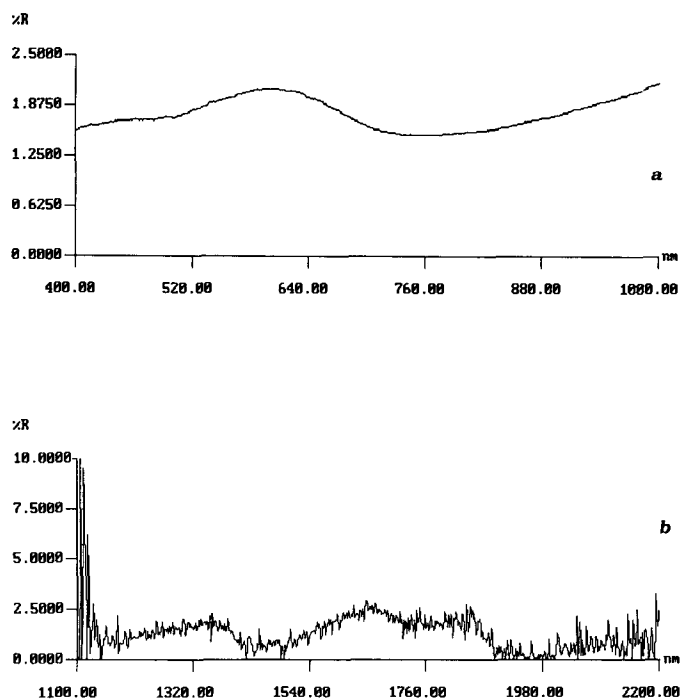
FIG. 2. Skin reflectance of a specimen of *P. m. marcuccii*; a and b as in Fig. 1.

optical fiber; in fact, about 2% of the overall radiation is lost, and this fact must be taken into due account when energy balance considerations are made. In spite of this drawback, we preferred to use the present apparatus owing to the wide radiation range which could be investigated, the quickness of the time required for the measurement, and consequently, the reduction in the trouble given to the animals.

The white light reference was $BaSO_4$, which has a good total mean diffuse reflectance ($R \approx 90\%$) in the entire range considered.[9,10] An 800 L/mm grating and a silicon detector were used for radiation in the range 0.40–1.05 μm, while the range 1.05–2.20 μm was covered with a 300 L/mm grating and a PbS cell as detector. Since our two electro-optical configurations are not very suitable for detecting radiation in the window 1.00–1.10 μm, a noise ten times higher is present in this range.

The reflectance spectra values were successively normalized with respect to the spectral distribution of sunlight at sea level. In other words, the solar spectrum at sea level is not flat, so that the energy input does depend on the wavelength. Accordingly, the solar spectral irradiance at sea level was calculated following the LOW-TRAN 7 AFGL program[11] and assuming, as a model, that the sun was at 30° from the zenith, the sky clear, and the relative humidity = 76.11%, with mean values for the main atmospheric gases and pollutants. Actually at sea level, particularly in rocky islets, the contribution of the infrared radiation is greater than that transmitted by the atmosphere, owing to the black-body emission from the sea and the rocks. However, at the wavelengths considered (≤ 2.2 μm) the black-body radiation contribution is negligible for our purposes.[12]

All experimental measurements were carried out at room temperature ranging from 19 to 21°C.

RESULTS AND DISCUSSION

The resulting spectra (Figs. 1 and 2) show that skin reflectance over the entire region investigated is low ("dark" lizards: mean value of reflectance = 1.7%; "light" lizards: mean value of reflectance = 3.1%) through all wavelengths examined (0.4–2.2 μm); the scans basically present a similar pattern. The reflectance peaks in the green-yellow region (550–575 nm) are due to the simultaneous effect of Tyndall scattering by guanine crystals, which occur in the guanophores of the dermis, and the absorption by the xanthophores, which act as a yellow filter.[3] In the NIR region the absorption peaks of the O-H groups in water are particularly evident at 1.45 μm and around 1.9 μm; also evident is the absorption around 1.7 μm due to the first overtone of the C-H stretching vibration. As for the other absorption bands, any attribution would be arbitrary, owing to the noise and the complexity of the material examined.

The skin of both kinds of lizards is a little more reflective in the near-infrared (0.8–2.2 μm) than in the visible (0.4–0.8 μm) portion of the spectrum. Furthermore, the spectra of "dark" and "light" lizards tend to coincide over 1.85 μm. We calculated the energy input, normalizing with respect to the solar irradiance at sea level (Fig. 3) and then integrating the reflectance spectra recorded for each specimen. An amount of 2% was also added to account for the quantity lost by our recording instrument; then the obtained values were multiplied by 0.90 (mean value for the total diffuse reflectance of $BaSO_4$). The energy input was calculated by subtracting values so obtained for reflectance from the total energy available at sea level (92.44 mW/cm²), as derived from our model.

Energy absorption is consequently high at all wavelengths examined in all lizards tested (overall mean:
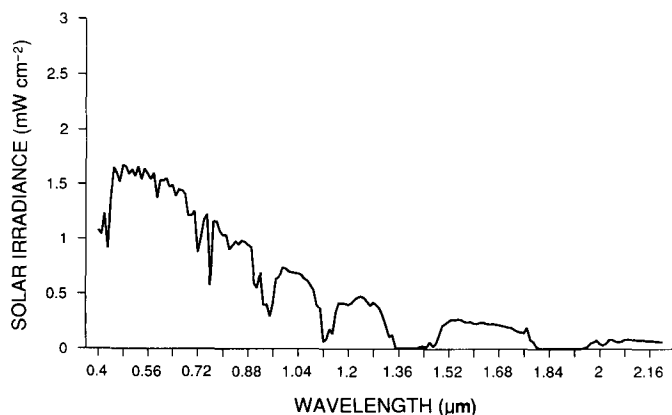
FIG. 3. Solar spectral irradiance at sea level as calculated following the LOWTRAN 7 AFGL program (see text).

"dark" lizards = 90.89 mW/cm², "light" lizards = 89.62 mW/cm²). Both lizard groups absorb more energy in the visible than in the NIR. This is due to the fact that solar irradiance is higher in the visible (59.3% of the total energy available) than in the NIR (39.3%). Nevertheless, a slight difference exists (from 0.69 to 2.13 mW/cm²) in the total energy input between "dark" and "light" lizards. However, it has to be noted that such a difference is not as evident as might be expected on the basis of a simple visual comparison. The slight difference found is consistent with the different overall chromatic pattern of the two phenotypes. In fact, "light" specimens differ from "dark" ones mainly in having a smaller amount of epidermal melanophores, while a thick continuous dermal stratum of hidden melanin-bearing cells occurs in both forms.

Furthermore, present reflectance values, and consequently the energy input, show a difference ($\approx 4\%$) in comparison to the values reported in the literature for this species,[7,13] which could be attributable to the different measuring system and to the more accurate method of calculating the energy input used here.

On the other hand, it is important to note that this discrepancy affects only the absolute reflectance values, while the difference in skin reflectance between the two lizards subspecies is almost the same (1.4% in this paper, 2% in Ref. 7).

In our opinion the data on the difference in energy input in the two phenotypes are likely to be regarded as being valid since: (1) the range of solar irradiance examined roughly corresponds to 98.6% of the solar energy available at sea level; (2) the pigments themselves, which are present in both phenotypes, affect the absorbance of wavelengths shorter than 2.5 μm;[14] and (3) in those lizards in which labile coloration occurs, color change typically alters reflectivity only in the 0.32–1.15-μm range.[15]

In conclusion, our results show that skin reflectance spectra of living animals can be easily and safely recorded without any injury to the animal; moreover, the method allows acquisition of information that could be lost when the tissue is excised from live animals. Another consideration, which arises from our data, concerns the following question: Can the small difference in energy input account for an overall heating, which is significant from a functional point of view? Of course, the response to this question involves biological considerations, which are beyond the purposes of the present article and will be treated elsewhere.

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