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Characteristics of a Microsporidium (Protozoa: Microspora) Infecting Grasshoppers (Orthoptera: Pyrgomorphidae) in Cape Verde, Africa

CARLOS E. LANGE*,[1] JORGE M. BRITO** and JOHN E. HENRY***

**Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Centro de Estudios Parasitológicos y de Vectores, Universidad Nacional de La Plata, Calle 2 No. 584, (1900) La Plata, Argentina,*
***Instituto Nacional de Investigacao Agrária, P.O. Box 128, Praia, Santiago, Cape Verde, and*
****Entomology Research Laboratory, Montana State University, and USDA, ARS, Rangeland Insect Laboratory, Bozeman, Montana 59717-0366, USA.*

ABSTRACT. A light and electron microscopic study was conducted of a microsporidium isolated from the grasshopper *Pyrgomorpha cognata* Krauss, 1877 collected in Santo Antão and Santiago Islands, Cape Verde. The evidence suggests that although there are some differences, such as tissues affected and size of spores, the organism appears conspecific with *Nosema pyrgomorphae* Toguebaye, Seck & Marchand, 1988, which was described from another species of the genus *Pyrgomorpha* Audinet-Serville, 1838 in Senegal. However, in addition to the differences in tissue specificity and size of spores, light microscopy studies also revealed some stages of the pathogen (uninucleate bodies and plasmodia) apparently not previously observed in *N. pyrgomorphae*.

Key words. *Nosema pyrgomorphae, Pyrgomorpha.*

GRASSHOPPERS of the genus *Pyrgomorpha* Audinet-Serville, 1838 are commonly found in the Sahel Region of Africa and the Cape Verde archipelago [4]. Recently, Toguebaye et al. [14] described a microsporidium, *Nosema pyrgomorphae*, from grasshoppers of that genus in Senegal. The definitive host remains unclear because in the description the authors mentioned two different species, *P. conica* (Olivier, 1791) and *P. bispinosa* (Walker, 1870), as hosts.

During a survey for grasshopper pathogens in Cape Verde, microsporidiosis was observed in *P. cognata* Krauss, 1877 from the islands of Santo Antão and Santiago. This paper presents the characteristics of the etiological agent responsible for those infections, with particular emphasis on a comparison with the microsporidium reported by Toguebaye et al. [14].

MATERIALS AND METHODS

Surveys for infected grasshoppers were conducted in Santo Antão, Sao Vicente, Santiago, Maio, Boa Vista, Sal, and Fogo Islands. Tissues were routinely examined for infection as fresh mounts with saline under phase contrast microscopy. Permanent preparations were air dried, fixed in methanol and stained with Giemsa as described by Wang et al. [16].

For transmission electron microscopy, tissues were fixed in 2.5% (v/v) glutaraldehyde buffered with 0.1 M cacodylate buffer, pH 7.4. Postfixation was in 1% (w/v) OsO_4 and was followed by dehydration in an ethanol series. Tissues were embedded in Spurr's resin and sections were stained with uranyl acetate followed by lead citrate and examined with a JEOL JEM CX electron microscope at 100 kV.

RESULTS

General observations. Infected grasshoppers were collected in Santo Antão and Santiago Islands. Natural prevalence of

[1] To whom correspondence should be addressed. Current address: Rangeland Insect Laboratory.

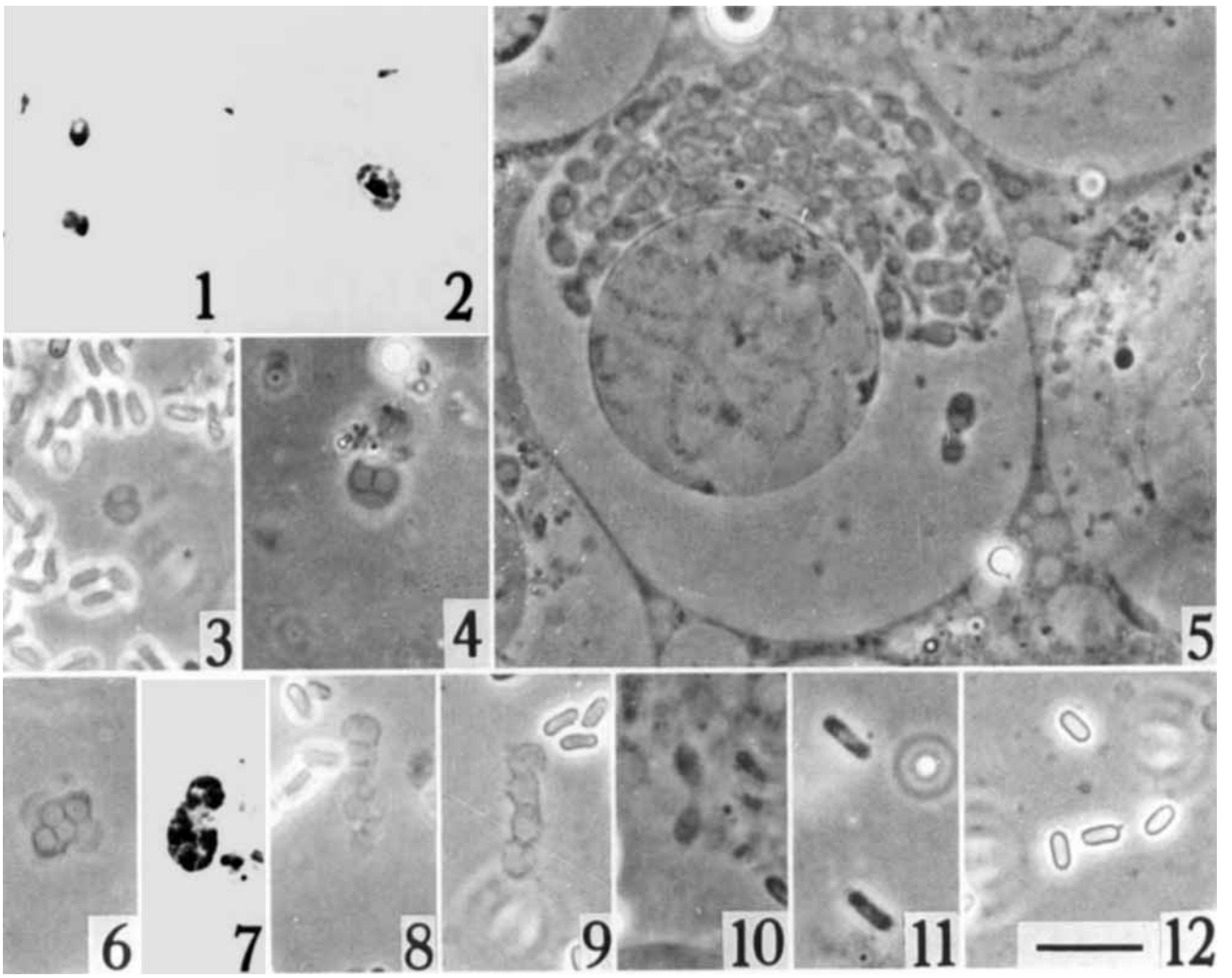
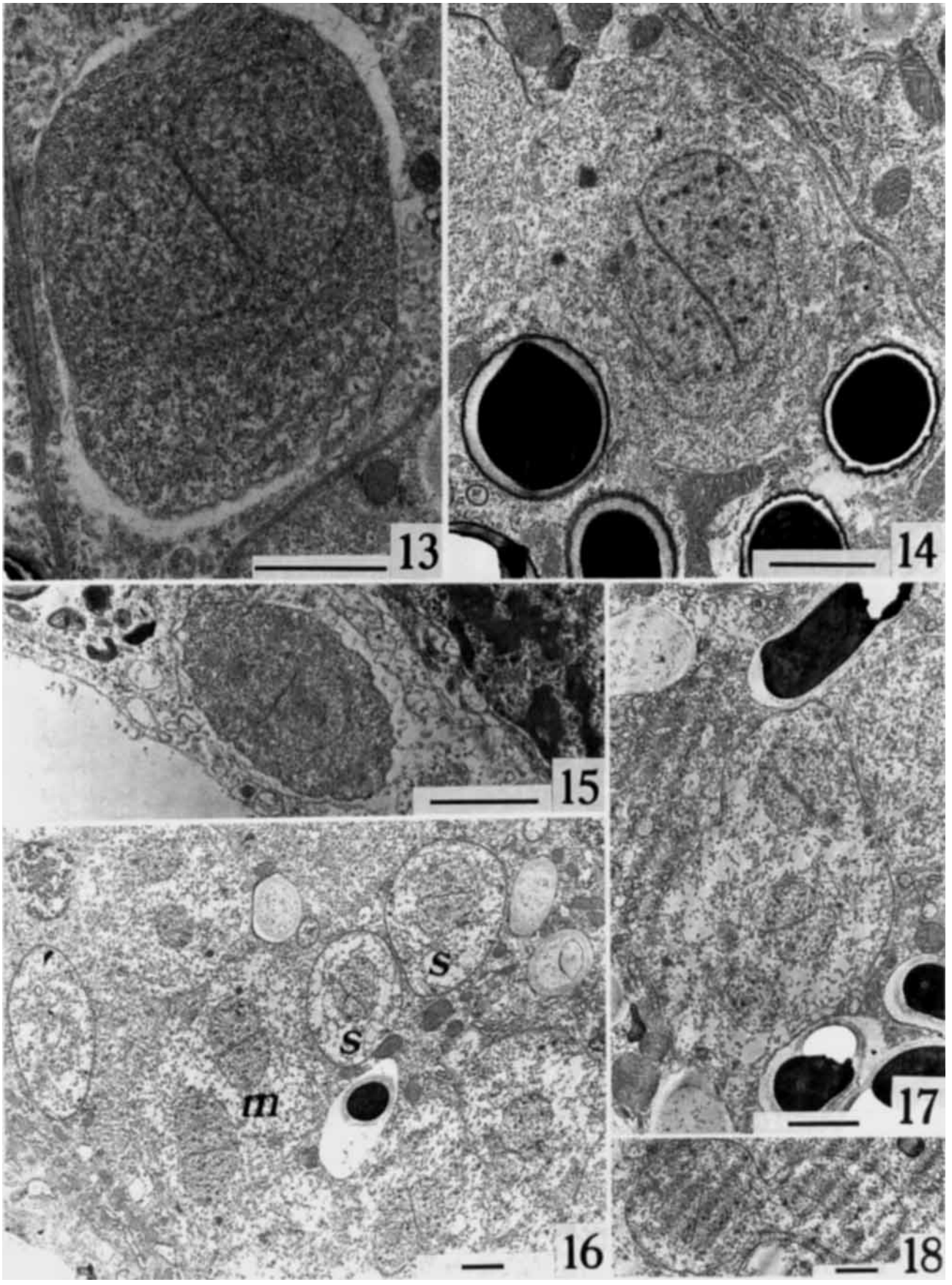


Fig. 1–12. Developmental stages of *N. prygomorphae* in Giemsa-stained smears (Fig. 1, 2, 7) and fresh mounts (Fig. 3–6, 8–12). **1**. Small uninucleate stages. **2**. Big stage undergoing intense nuclear activity. **3, 4**. Rounded diplokaryotic stages. **5**. Diplokaryotic stages in the cytoplasm of an oocyte. **6**. Tetranucleate diplokaryotic cell. **7**. Plasmodium. **8**. Elongate plasmodium. **9**. Moniliform plasmodium. **10**. Binary fission of a tetranucleate diplokaryotic cell. **11**. Sporoblasts. **12**. Spores. Bar = 10 μm.

infection among late nymphal instars (fourth and fifth) and adults from Santo Antão was 34% (n = 62). Also, infections were found among grasshoppers reared in the laboratory for two generations. Disease prevalence among reared grasshoppers reached 44%, but increased mortality due to disease was not evident. Infections were equally prevalent in both green and brown color phases of the host. Specimens of *Acorypha clara* (Walker, 1870) (n = 62), *Acrotylus longipes* (Krauss, 1907) (n = 27), *Acrotylus patruelis* (Herrich-Schäffer, 1838) (n = 10), *Aiolopus simulator* (Walker, 1870) (n = 52), *Anacridium melanorhodon* (Walker, 1870) (n = 58), *Diabolocatantops axillaris* (Thunberg, 1815) (n = 116), *Oedaleus senegalensis* (Krauss, 1877) (n = 126), *Pseudosphingonotus savigni* (Saussure, 1884) (n = 21), and *Trilophidia conturbata* (Walker, 1870) (n = 8) collected from Santo Antão and Santiago Islands were not infected. Infected insects exhibited no gross signs of infection. Examination revealed that the salivary glands and gonads of both sexes were affected. Normally, infections were heavier in salivary glands than in gonads. Heavily infected glands appeared hypertrophied, were darker in color and were more granular than normal glands. Diagnosis of infections in gonads was possible only by observation of the parasite's developmental stages under the compound scope.

Light microscopy studies. Most of the developmental stages observed were diplokaryotic, although two stages were observed which were not diplokaryotic. These were small, mostly spherical uninucleate bodies (Fig. 1) and big, spherical stages apparently undergoing intense nuclear activity or rearrangement (Fig. 2). The most frequently observed developmental stages were binucleate diplokaryotic cells in both glands and gonads. They tended to be spherical in glands (Fig. 3, 4) while mostly fusiform in oocytes, in which they frequently occupied much of the host cell cytoplasm (Fig. 5). Tetranucleate diplokaryotic cells also were common (Fig. 6). Plasmodia (Fig. 7), and sometimes elongate (Fig. 8) or even moniliform plasmodia (Fig. 9), were observed only in glands. Binary fission of tetranucleate cells to form binucleate diplokaryotic stages was common (Fig. 10). Binucleate sporoblasts were usually longer than spores and not refringent (Fig. 11). Fresh ovocylindrical spores (Fig. 12) measured (mean ± standard error) 3.4 ± 0.01 μm by 1.2 ± 0.004 μm (n = 50).

Electron microscopy studies. Similar developmental stages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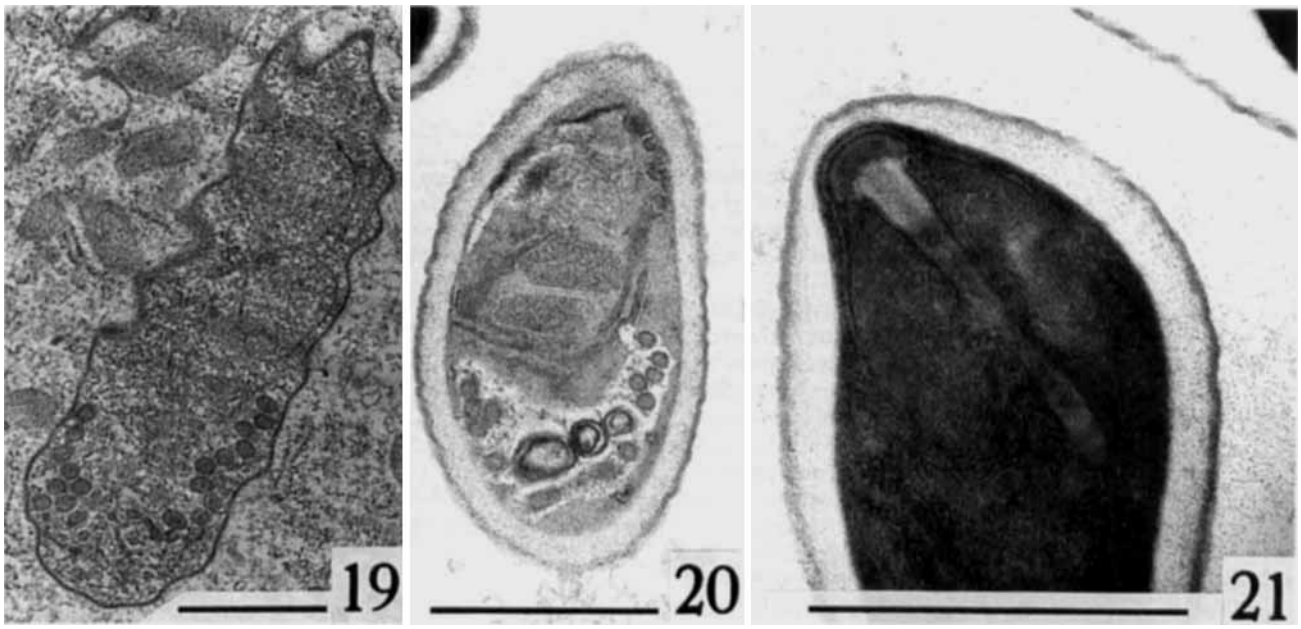

Fig. 19–21. Ultrastructure of a sporoblast and mature spores of *N. pyrgomorphae*. **19**. Diplokaryotic sporoblast showing irregular outline and formation of polar filament. **20**. Diplokaryotic spore. **21**. Partial view of mature spore showing anchoring disc, manubrium, and lamellar polaroplast. Bars = 1 μm.

were seen in both glands and gonads. Development was in direct contact with the host cell cytoplasm, although in some instances a space was observed between the parasite's plasmalemma and the surrounding host cytoplasm (Fig. 13). The nuclear condition was always diplokaryotic. Large binucleate meronts mostly were spherical in glands (Fig. 14) while in gonads they appeared more fusiform (Fig. 15). The cytoplasm of meronts appeared dense, with an abundance of both rough endoplasmic reticulum membranes and free ribosomes. Tetranucleate meronts were common (Fig. 16). Stages in sporogony were distinguished from those in merogony by a thickened outer membrane. Plasmodia in transition from merogony to sporogony were present (Fig. 17). Apart from the thicker plasmalemmas and less dense cytoplasm, sporonts (Fig. 16) did not differ in appearance from meronts. Binary fission was common during sporogony (Fig. 18). Sporoblasts (Fig. 19) were binucleate, elongate and with irregular outlines. Mature spores (Fig. 20) also were diplokaryotic with typical lamellar polaroplasts (Fig. 21) and a maximum of eight coils of the polar filament.

DISCUSSION

The results obtained indicate that *N. pyrgomorphae* was the etiological agent responsible for the infections observed in grasshoppers from Santo Antão and Santiago, and provide additional information on the characteristics of the microsporidium. Although a comparison at the light microscopy level is not possible because the original description of *N. pyrgomorphae* by Toguebaye et al. [14] did not include such a study, at the ultrastructural level the microorganisms were indistinguishable. Both show the organism to be monomorphic, diplokaryotic and apansporoblastic, and the spores have the same ultrastructural features (number of polar filament coils, nuclei arrangement, type of polaroplast). However, the observations of the Cape Verde isolate under light microscopy indicated that uninucleate and plasmodial stages were present during the developmental cycle of *N. pyrgomorphae*. This is difficult to determine with accuracy by working only with sectioned tissues under electron microscopy.

Probably, the most significant difference observed between the microsporidiosis in Senegal and that in Cape Verde was the tissue specificity. Toguebaye et al. [14] reported that *N. pyrgomorphae* developed in the epithelial cells of the digestive tract, adipose tissue and in skeletal muscle fibres. In insects from Cape Verde, the parasite was seen only in salivary glands and gonads of both sexes. This is an interesting phenomenon not only because of the biological connotations regarding host-parasite relationships but also from a taxonomic point of view. In the past, many similar microsporidia were considered as different species solely on the basis of different tissue specificity. Hazard and Lofgren [6] demonstrated that a *Nosema* sp. infecting mosquitoes developed in different tissues depending on the host species and concluded that tissue specificity was not a reliable criterion for the separation of species in Microspora. In the case of *N. pyrgomorphae*, a possible explanation for the observed differences could be due to different hosts. In their description, Toguebaye et al. [14] worked with either *P. conica* or *P. bispinosa*, species that often are virtually indistinguishable from each other [9]. Although *P. cognata* also is extremely similar to *P. conica* and *P. bispinosa*, we were able to distinguish it using information provided by Kevan [11] and Hsiung and Kevan [9]. The possibility also exists that development of the pathogen in a similar but different host species could be the reason why spores in *P. cognata* infections were smaller, particularly in their width, than those of *N. pyrgomorphae* in *P. conica* or *P. bispinosa*. This

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Fig. 13–18. Transmission electron micrographs of *N. prygomorphae*. **13**. Diplokaryotic meront showing a space between some parts of the plasmalemma and the host cell surrounding cytoplasm. **14**. Rounded diplokaryotic meront. **15**. Slightly fusiform diplokaryotic meront in gonads. **16**. Tetranucleate diplokaryotic meront (m) and diplokaryotic sporonts (s). **17**. Diplokaryotic stage in transition from merogony to sporogony showing six nuclei. **18**. Sporont undergoing binary fission. Bars = 1 μm.

would be in accord with the concept presented by Hazard et al. [7] that spore size and shape also is not reliable for differentiating species. Spore plasticity has been reported by Blunk [2], Walters [15], Sedlacek et al. [13], Armstrong et al. [1], Mercer & Wigley [12], and Hayasaka & Kawarabata [5].

Kawarabata & Hayasaka [10] provided evidence of microsporidian strains and the possibility exists that the infections in grasshoppers from Senegal and Cape Verde were caused by different strains of *N. pyrgomorphae*. Cross infection experiments using hosts and pathogens from both Senegal and Cape Verde need to be undertaken to answer this prospect.

Presence of infections in oocytes and the development of infected insects in two generations strongly suggests that vertical transmission must play a significant role in the maintenance of *N. pyrgomorphae* infections in natural populations of grasshoppers. The lack of an easily achieved oral horizontal transmission would not be unexpected because the increase in prevalence from 34% in the field to 44% in caged grasshoppers over two generations does not seem to be significant. This will be clarified as soon as a colony of *P. cognata* is available for inoculation experiments. The almost complete lack of mortality in grasshoppers of generations one and two, apart from those dying as older adults, would suggest that *N. pyrgomorphae* is, as most parasitic protozoans of insects are [3, 8], a pathogen of low pathogenicity.

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Action Spectra for Step-Up Photophobic Response in *Blepharisma*

TATSUOMI MATSUOKA,[1] SETSUKO MATSUOKA, YUMIKO YAMAOKA, TOSHIHIKO KURIU, YOSHIYA WATANABE, MIYUKI TAKAYANAGI, YOJI KATO and KOJI TANEDA

Department of Biology, Faculty of Science, Kochi University, Kochi 780, Japan

ABSTRACT. The cells of *Blepharisma* which possess red pigment (blepharismin) show step-up photophobic response (temporal ciliary reversal induced by a sudden increase in light intensity). Bleaching of the cells by cold shock raised a threshold light intensity for the response. Oxidation of red pigment that produced blue pigment did not raise the threshold for the response. The action spectrum for the step-up photophobic response of the cells which possess normal red pigment had peaks at about 580, 540 and 490 nm, a value which coincided with peaks of an absorption spectrum of the red pigment. The absorption spectrum of oxidized pigment (blue pigment) shifted 20 nm toward infrared light. The action spectrum for the response of the cells which possess blue pigment also shifted 20 nm toward infrared light. Results suggest that red pigment might be involved in the step-up photophobic response.

Key words. Blepharismin, ciliary reversal, photoreceptors, photoresponse.

SOME unicellular organisms, especially colored cells such as *Chlamydomonas* [2], *Euglena* [1], *Stentor coeruleus* [14], *Blepharisma* [9–11] and *Paramecium bursaria* [5, 13] show a remarkable photoresponse in response to a step up or step down in light intensity. Some colorless cells also respond to light [6, 7]. A basic question in studies on photoresponses is what kinds of photoreceptor pigments mediate the photoresponses. In order to speculate on functional photoreceptor pigments, the action spectrum for the photoresponse can be compared with an absorption spectrum of pigment endogeneously contained in an

[1] To whom correspondence should be addressed.