SOME ADAPTATIONS OF THE ANTHOCEROPHYTE *Megaceros pellucidus* (Colenso) E.A.Hodgs. TO EXTREMELY LOW LIGHT ENVIRONMENTS

A thesis presented in partial fulfilment of the requirements for the degree

Of Master of Science

In

Plant biology

At Massey University, Palmerston North,

New Zealand

Roger Lionel Sloane Watkins

2002

Errata

p2 Mache (1973) should be Marche & Loiseaux (1973); Citations referencing; Aro, should not be underlined.

p5/6 Smith et al = Smith & Griffiths (1996).

p18 Line 3; delete Campbell ; 1984 retain 1995 as the one citation.

p20 Raemaekers (1987) should be Raemakers and Longwith (1987).

p21 3.3.1 line 8 the □ should read ±.

p29 Line 6 .."At the of which"...should read, "At the end of which"..

p37 Table number 4.1 should read 4.0 not 4.1.

p39 Figure 4.4 suffers from heavy pixellation.

p41 Table number 4.2 should read 4.1 not 4.2.

p42 Table number 4.3 should read 4.2 not 4.3.

p43 Table number 4.4 should read 4.3 not 4.4.

p44 Table number 4.5 should read 4.4 not 4.5.

p54 Figure 5.2 caption .."white light or blue light".. should read, .." white light. Blue light > 3 µmoles $m^{-2}s^{-1}$ produces a similar result."

P55 Figure 5.5 the caption should read; "Micrograph (x100) of a transverse section across an *M. pellucidus* thallus "after 24 h in light of ~3 µmoles $m^{-2}s^{-1}$ showed the chloroplasts aligned on the periclinal walls".

p60 Figure 5.11 chloroplast mis-spelt in the second figure, cluster caption.

p61 Figure 5.12 add, □ = 3;..

p78 Line 10/11, Kagawa & Wada1, 2002;.. should read Kagawa & Wada, 2002;

p80 Should be Loomis and Connor (1992).

p84 Citations referencing; Aro, should not be underlined.

p90 Line 10 Tlalka & M.., 1999; should read; Tlalka & Fricker,1999;

p97 Burr, F.M. (1968) should be Burr, F.A. (1968).

ABSTRACT

The New Zealand Anthocerophyte *Megaceros pellucidus* (Colenso) is found in wet, cool temperate rain forest and is associated with extremely low light habitats (0.5-7 μmoles photons m^{-2} s^{-1}). The light available to *M. pellucidus* was found to be only 0.2% of the overhead crown canopy light and was heavily attenuated after passing through many leaf canopies. This thesis shows that the photon flux density in these extremely low light habitats can be augmented by two additional light sources, sunfleck light, especially at midday, and light reflected from adjacent water surfaces, such as rivers or ponds, as the sun's incident ray path angle diminishes late or early in the day.

This thesis looks at some of the strategies *M. pellucidus* uses to survive in its low light habitat and, in adapting to acquire such sensitivity to low light parameters, how *M. pellucidus* protects itself from photoinhibition if exposed to high white light of more than 140 μmoles photons m^{-2} s^{-1} or blue (470 nm) light of more than 3 μmoles photons m^{-2} s^{-1}.

The chloroplast position in *M. pellucidus*, when in its normal habitat, was found to retain an expanded form situated on the periclinal cell wall proximal to the light source (an epistrophe position). When thallus tissue sections of *M. pellucidus* were irradiated with blue light of more than 3 μmoles photons m^{-2} s^{-1} or white light of more than 140 μmoles photons m^{-2} s^{-1} the chloroplast shrank dramatically and assumed a position on anticlinal walls (a parastrophe position). Red (662 nm) light of less than 130 μmoles photons m^{-2} s^{-1} or darkness had no obvious effect on the morphology epistrophe chloroplasts, but this treatment resulted in the chloroplasts expanding and moving back to the epistrophe position after irradiation by blue or high levels of white light.

Based on the rate of volume change occasioned when the chloroplasts were irradiated with blue, white, red light or darkness it was concluded that a water flux was induced across the membranes of the various intracellular organelles that depended on the wavelength of the light and the photon flux density.

Various concentrations of polyethylene glycol-20 (PEG) were used as an osmoticum and induced chloroplast shrinkage to an extent and at a rate similar that induced by blue light. Red (662 nm) light of 130 μmoles photons m^{-2} s^{-1}, was observed to expand the chloroplast volume against the osmotic gradient, while darkness had no effect.

A comparison of transmission electron microscope (TEM) micrographs taken of both blue / high light conditions and dark or red irradiated chloroplasts show differences in thylakoid membrane architecture, the dark-exposed samples having a loose open form with pseudograna and greater areas of stroma compared to the blue and high light samples that showed a tight compression of the thylakoids and very reduced areas of stroma. Large numbers of starch granules were apparent in all but the blue irradiated TEM micrographs. Examination of the micrographs showed there were obvious differences between the size of the starch granules (TEM, x7800, micrographs having starch granules with a dark to light ratio of 2.165) as well as in the texture and density.

ACKNOWLEDGEMENTS

Firstly I wish to extend my gratitude and thanks to my mentor and initial supervisor Dr. Simon Brown not only for his supervision but also for his friendship, personal support under sometimes-trying situations and his unfailing enthusiasm for this project. Also thanks to my co-supervisors; Dr. Heather Outred for her encouragement, hospitality and help, and to Dr. Al Rowland for his advice and the extensive joke entrepôt.

My thanks also to Professor Paula Jameson for her support, Professor Roderick Thomas for his initial advice and to the various staff members and technicians of the Institute of Molecular Biosciences who were always friendly and willing to help.

I count myself in being fortunate in having received the expertise, friendship and help of two very charming ladies; Elizabeth "Liz" Nickless with the Confocal microscope and microphotography and Judy Englebrecht for her help in the preparation of samples and use of the CRI transmission electron microscope .

My thanks also to the Dept. of Plant Health for the loan of both the LI-COR 185 and LI-COR 250 light measuring instruments as well as to the Massey electronics workshop for the modification and supply of the optic pen and light projector source.

My thanks to my wife Barbara who had to put up with my very late nights, absences from home and the piles of books and papers spread throughout the house during the course of this study.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS USED IN TEXT

2n	diploid
CCM	carbon concentrating mechanism
Ch Thy	channel thylakoid
Chl *a*	chlorophyll *a*
Chl *b*	chlorophyll *b*
Chl E	chloroplast envelope
Cw	cell wall
Cyt	cytoplasm
DMSO	dimethyl sulfoxide
ER	endoplasmic reticulum
Gs	grana stack
H	"honeycomb" effect
L/S	longitudinal section
LCP	light compensation point
LED	light emitting diode
LHCP	light harvesting complex protein
LRW	London Resin Co. Ltd. (white resin)
LSP	light saturation point
m.y.a.	Million years ago
MIP	major intrinsic protein
Ml	middle lamella
n	haploid
nm	nanometer
NMR	nuclear magnetic resonance
PAR	photosynthetically active radiation
PEG	polyethylene glycol-20
PFD	photon flux density
PG	plastoglobuli
Pl	plasmalemma
PSII	photosystem II
PSI	photosystem I
P_s	photosynthesis
Q_{10}	respiratory quotient
Rubisco	ribulose bisphosphate carboxylase / oxygenase
SEM	scanning electron microscope
SG	Silicon Graphics
SOD	super oxide dismutase
Spp	species
St G	starch granule
St	stroma
T/S	transverse section
TEM	transmission electron microscope
Thy O	thylakoid (open ended)
Thy S	thylakoid strands
Thy	thylakoid
TIP	tonoplast intrinsic protein
Ton	tonoplast

Voxel	$pixels^3$
V	query vacuole
X/S	cross section

Chapter 1

Introduction

Ecologically plants can be classified into sun or shade plants depending on the degree of genotypic and/or phenotypic adaptability to a particular light intensity (Bjorkman, 1981; Bjorkman & Holmgren, 1963; Boardman, 1977).

In areas subject to extreme shading only a few specialized plants are able to survive. The plants associated with these sites are mainly Bryophytes, Anthocerophytes and Pteridophytes. Generally all of the extreme shade areas, on which the various varieties grow, are cool and well supplied with water. The dry heavily shaded areas have few plants growing on them. The flora of these extremely shaded positions appear to have adapted to these low light situations by modifying and changing their physiological, anatomical and metabolic functions, to a greater or lesser extent.

Shade plants have their photosynthesis (P_s) saturated by relatively low photon flux densities (PFD) (light saturation rate of CO_2 uptake ranged from 2.1-3.1 mg CO_2 dm^{-2} h^{-1} for shade species compared to high light species which ranged from 21-36 mg CO_2 dm^{-2} h^{-1}) whereas full sun plants have a higher level of P_s since they have higher light saturation rates (Bjorkman, 1968)). The light is utilised within the chloroplasts that are able to function over a very wide range of light environments, (xerophytes in full sun >2500 μmoles photons m^{-2} s^{-1} to shade recesses in rainforest floors < 5 μmoles photons m^{-2} s^{-1}) (Barbour *et al.*, 2000; Bjorkman & Holmgren, 1963; Chazdon *et al.*, 1996; Etherington, 1982; Nobel, 1991; Osmond *et al.*, 1987).

The extreme shade plants are exceptionally efficient in their P_s ability; a ratio between the available PFD and the level of photosynthesis, the P_s efficiency of the shade plants is greater than the full sun plants (Böhning & Burnside, 1956). The extreme shade plant habitats have ample water, cooler temperatures than the sun exposed sites, adequate CO_2 (an average CO_2 concentration of 360 ppm at the forest floor level in a Queensland rainforest) (Bjorkman, 1981), but are limited by light,

thus any survival strategy must be directed towards some form of light enhancement or a more efficient metabolism (Khurana, 1998).

Light, in respect to shade plants, is the major limiting resource and requires an optimal cost benefit strategy (Grime, 1981; Hodgson *et al.*, 1999; Larcher, 1995; Rincon & Grime, 1989).

Shade plants and aquatic plants utilise a combination of strategies such as extended light harvesting antennae and use of alternative light wavelengths, changes in light harvesting chloroplast protein (LHCP) ratios (Grignon, 1999), changes in photosystem I : photosystem II (PSI: PSII) ratios (Boardman, 1977), light magnification and optic path modification (Vogelmann & Bjorn, 1986), changes in cellular ultra-structure (Chow *et al.*, 1982) and a wide range of gross morphological changes to achieve an ultimate light utilisation efficiency (Aro, 1982).

Since deep shade plants are genotypically adapted for survival in low light situations any exposure to high light could seriously compromise their survival (Boardman, 1977). Shade plants have adjusted to low light levels by acquiring low light compensation points (LCP), where respiratory CO_2 production equals P_s CO_2 uptake (Bjorkman, 1981).

In a shade plant the amount of light required to attain a P_s light saturation, where any further increase in PFD will not produce any further increase in P_s, is usually small for example, Mache (1973), estimated a value of 2-3 klux in *Marchantia polymorpha*. In light response curves of CO_2 uptake *vs* time with set PFD the efficiency of P_s can be seen when the slope of the curve is observed, the steeper the slope the more efficient the P_s. In shade plants the initial slope is generally very steep with a clearly defined saturation point and associated plateau in which no further P_s takes place (Bjorkman & Holmgren, 1963; Boardman, 1977) (Figure 1.1).

Photoinhibition occurs when light quanta absorbed exceeds that used for P_s, and if leaves cannot dissipate the extra energy harmlessly, molecular and biochemical damage will eventually occur as a result of excessive levels of free radicals (the oxide anions; O_2^-, HO_2, H_2O_2). Moderate excesses of free radicals are rendered safe by being scavenged and oxidised by such enzymes as superoxide dismutase (SOD) and peroxidases (Ishikawa *et al.*, 1993). Shade plants are more susceptible to

photoinhibition from high PFD levels because of their low light saturation point. So shade plants with adaptations to enhance light capture and optimize P_s, must also include mechanisms to reduce the likelihood of photo-damage (Boardman, 1977).

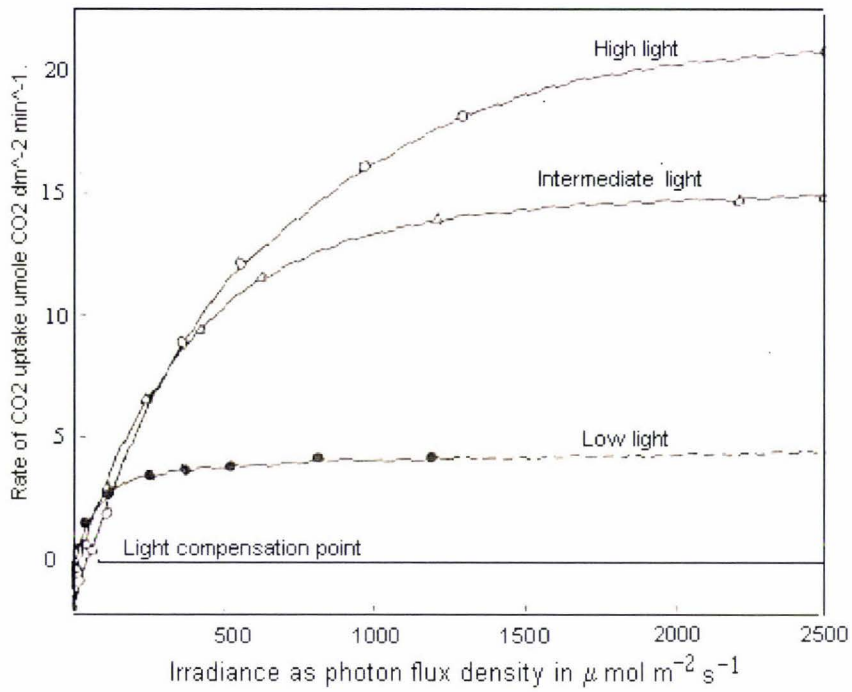

Figure 1.1. An example of light saturation curves, as exhibited by *Atriplex patula* grown at high light (20mW cm^{-2}) intermediate light (6.3mW cm^{-2}) and low light (2mW cm^{-2}) from (Bjorkman *et al.*, 1972).

Some strategies that have evolved in shade plants to minimize photoinhibition and bleaching are largely mechanistic. Changes in chloroplast volume (Ivanchenko *et al.*, 1980; McCain & Markley, 1992; McCain, 1995; McCain, 2000; Nobel, 1968) reducing surface area, and chloroplast movement that creates a minimal surface exposure to the light source (Haupt, 1982; Haupt, 1999; Haupt & Hader, 1994), a compaction of thylakoids reduces the LHCP's and electron chain sensitivity (Anderson & Aro, 1994; Anderson *et al.*, 1988; Bischof *et al.*, 1999; Lichtenthaler *et al.*, 1982; Vaughn *et al.*, 1992), and changes in light ray paths and photon scattering that disperses excess light energy (Vogelmann, 1993; Vogelmann & Bjorn, 1986; Vogelmann *et al.*, 1996).

Generally the majority of shade plants possess leaves that are thinner with greater surface area, with larger chloroplasts and have higher chlorophyll levels than the leaves of sun plants. The ratios of chlorophyll *a* (Chl *a*) to chlorophyll *b* (Chl *b*) drop, shade plants having higher proportions of Chl *b* (Anderson *et al.*, 1973; Chazdon *et al.*, 1996).

Ultrastructure investigations of shade plant chloroplasts show that thylakoid architecture differs from that of sun plant chloroplasts. Thylakoids of shade plant[1] chloroplasts have large irregularly arranged stacks (grana stacks of up to 100 thylakoids per granum) (Anderson *et al.*, 1973). This irregular arrangement would optimize the harvesting of weak diffuse light. In contrast the thylakoid architecture in chloroplasts exposed to high PFD is characterized by an arrangement of very condensed compact stacking with a paucity of visible stroma (Anderson & Aro, 1994). This arrangement suggests a sheltering pattern with the external units creating a shading effect on the more interior platelets.

Any shift in the proportions of stroma lamellae to grana stacks, in comparison between sun and shade plants is not evident but the length of the shade plant stromal lamellae is much shorter (Chow, 1999; Chow *et al.*, 1990). The ratio of appressed thylakoid regions between the stroma lamellae and the grana was 3:2, in the shade plant *Alocasia*, compared to 1:4, in a Spinach sun plant (Anderson *et al.*, 1973). In *Helianthus* it was found that the thylakoids in contact with the stroma surface-to-volume ratio, did not change relative to that of a high-irradiance control but remain significantly lower than that of a low-irradiance control. The change in the ratio of appressed thylakoids to thylakoids in contact with the stroma occurred within a relatively short time (5 min) with low PFD exposure and indicated a broadening and shortening of the appressed thylakoid stack (Wheeler & Fagerberg, 2000).

It appears that the sun/shade acclimatization process of the chloroplast thylakoids is mostly driven by changes in photon flux densities and not by specific spectral band-dependent receptors (Ghoshroy & Fagerberg, 1998). Although changes in the thylakoid distribution can be compacted in *M. pellucidus* by; light of PFDs > 3 μmoles photons m^{-2} s^{-1} in the blue (460-480 nm) waveband and expanded by red light (660-680 nm) of PFDs of up to 130 μmoles photons m^{-2} s^{-1} .

[1] Vascular plants

Anderson proposed that a more efficient collection of light quanta would result with any increase in Chl *b* and its associated LHCP's and it is this increase that is responsible for the expansion in the thylakoid shade orientation (Anderson *et al.*, 1973). A similar thylakoid expansion and similar stroma lamellae can be seen in *M. pellucidus* chloroplasts (section 5.7). However stroma diminishment in shade plants does not occur in *Megaceros*, rather the reverse and the extent of this is shown in Figure 5.21.

The sample plant that was chosen for this thesis, the Anthocerophyte *Megaceros pellucidus* (Colenso) E.A.Hodgs. (Chapter 2) is an extreme shade plant and common to all of the four sample sites (Chapter 4) and is found growing in light ranging from 0.1 up to 7 μmoles photons m^{-2} s^{-1}. The range, 0.1-7 μmoles photons m^{-2} s^{-1}, is referred to, in this text, as the habitat light.

While the chlorophyll content in shade plant chloroplasts is generally higher, relatively, than that found in sun plants, the chloroplast number per unit area of leaf is very much reduced (Chazdon *et al.*, 1996; Chow *et al.*, 1990). This has been explained on a cost / benefit analysis, where there exists a diminishing return for increasing chloroplast concentration.

Measurements made of the PFD at four *M. pellucidus* sites (Chapter 4) show a extremely low level of available light PFD (0.07 moles photons m^{-2} day^{-1}), an attenuation of 99.84 % of a crown canopy PFD irradiance of 42.93 moles photons m^{-2} day^{-1} (Chapter 4). Bjorkman (1981) concluded that in extremely low light situations a further doubling of the chloroplast number will only increase absorption by 3-6%, an energy budget deficit.

The size of the single chloroplast in the cells of *M. pellucidus* was found to be ideal for microscopy and volume measurement and a precedence of using the *M. pellucidus* chloroplast has previously been established: such as chloroplast gross morphology and ultrastructure (Valentine, 1984), the phylogeny and division of chloroplasts (Burr, 1968). Vaughn published a comprehensive review of the Anthocerophyta ultrastructure, incorporating much of his own research (Vaughn *et al.*, 1992). The evidence of carbon concentration in the Anthocerophyta species chloroplast pyrenoid and the associated Rubisco levels was described by Smith

(1996). Molecular genetics, especially RNA editing, was investigated by Yoshinaga (1997).

However, in all of the Anthocerophyte literature surveyed, there were only brief references to light stimulus and no actual quantification of light flux was made. As will be seen in these results any plant cellular ultrastructure experimentation, involving light, should include the light wavelength and PFD used. Earlier work has been done investigating the chloroplast movement in thallose Bryophytes by (Britz, 1979; Hader, 1987; Haupt, 1982; Haupt, 1999; Haupt & Hader, 1994; Kendrick & Kronenberg, 1994) and many others. But apparently little research has been conducted on volume changes to chloroplasts in response to light. (Chapter 2).

This work observes the effects of light at various PFDs, specifically red and blue wavelengths, on the chloroplast and its ultrastructure, of *M. pellucidus*. All previous workers have only briefly commented that the *Megaceros* chloroplast, orientation and size was subject to considerable variation when exposed to light (Burr, 1968; Valentine, 1984; Vaughn *et al.*, 1992) but there were no recorded qualitative or quantitative measurements taken.

During the exposure to high PFDs or blue light the volume of the *M. pellucidus* chloroplast was observed to shrink (Chapter 5). However the total cell volume did not appear to decrease at the same time, although no measurements of total cell volume were taken.

It could be assumed therefore, that the constituents of the cell are retained and simply relocated into other organelles or extended within the cytoplasm as a reconstituted product. This appears very similar to an osmotic reaction and has been commented on and investigated by a number of workers using species other than extreme shade plants (Gupta & Berkowitz, 1988; McCain & Markley, 1992; McCain, 1995; McCain, 2000; Robinson, 1985; Weiss, 1996). In this work the tissue of *M. pellucidus* was immersed in an osmoticum and a similar contraction to high PFD and blue light was seen to occur (section 5.6).

In the low light conditions of its natural habitat, the various species of Anthocerophyta collected, exhibited a maximum of "chloroplast to light exposure" both in surface area and in spatial orientation. This chloroplast characteristic was

first reported by Bohm in 1856 (Kendrick & Kronenberg, 1994) who commented on the change that occurred in chloroplast orientation in response to light conditions.

Chloroplasts respond to high light intensity by diminishing their exposed surface area (the major plane of the chloroplast lying parallel to the path of incident light) whereas in low light intensity their surface area tends to be maximized, (the major plane of the chloroplast at right angles to the path of incident light). This chloroplast phenomena was also investigated by Frank (in 1871) and Stahl (in 1880) (Haupt, 1982; Kendrick & Kronenberg, 1994) who commented that the orientation of these organelles appeared to depend on the direction of the light source and the intensity of this incoming light.

Chloroplast migration, in response to light, is common to the majority of plants (Britz, 1979; Haupt, 1982; Haupt, 1999; Haupt & Hader, 1994). Three species, in particular, *Selaginella, Mougeotia and Lemna,* have been intensively researched and the chloroplast arrangements, in response to light of various intensities, has been described (Britz, 1979) (Figure 1.2).

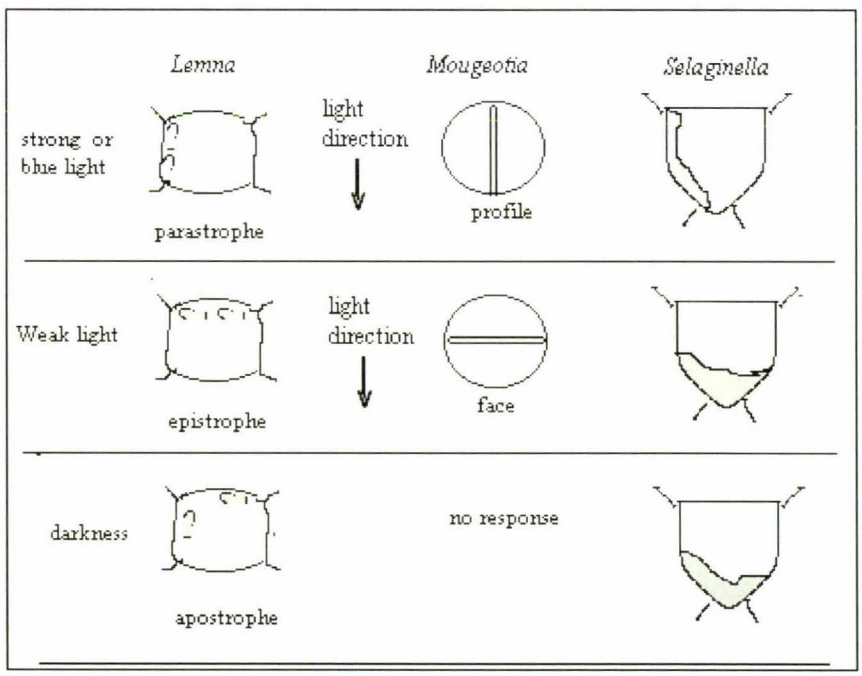

Figure 1.2. Diagrammatic representation of the various chloroplast arrangements in three plant species. The direction of the incident light is indicated by the arrows. The terms parastrophe, epistrophe and apostrophe have been used within this thesis (Adapted from Britz 1979).

The chloroplast arrangement for the species *Lemna*, as illustrated in Figure 1.2, appears analogous to *M. pellucidus,* detailed in this thesis, and the arrangement terminology as used by Britz (1979) will be used in this thesis.

1.1 Hypothesis

That the extreme shade tolerant plant, *Megaceros pellucidus* (Colenso) E. A. Hodges), has a number of co-ordinated, anatomical and physiological characteristics that enable it to survive in the extreme low light situations in which it is found.