THE EFFECTS OF LIGHT INTENSITY AND BLUE, GREEN AND RED WAVELENGTHS ON MATING STRATEGIES IN THE SNOW ALGA, *CHLOROMONAS* SP.-D, FROM THE TUGHILL PLATEAU, NEW YORK STATE

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ABSTRACT

In this study, four strains of the snow alga, *Chloromonas* sp.-D, were isolated from the Tughill Plateau, Whetstone Gulf State Park, NY, to study mating strategies under different light conditions. Experiments were conducted in white acrylic test tube holders that were placed in growth chambers with controlled light, temperature and photoperiod. Two light sources were used, Wide-Spectrum (WS) and Cool-White (CW), and the effects of blue, green and red light were tested by covering the holders with corresponding cellophanes. Cells were fixed with OsO_4 and observed and tabulated using Zeiss phase-contrast microscopes. Using similar light intensities, blue light regimes produced more matings than green light regimes in four of four trials (two were significantly different, $P<0.05$), and blue light regimes produced more matings than red light regimes in seven of eight trials (three were significantly different, $P<0.05$). There were no trends in mating when green and red light regimes were compared in three trials. A photon light intensity of 200 μmol m^{-2} s^{-1} produced the most mating under both WS and CW regimes, but more mating occurred under CW in all intensities tested. When red light regimes of higher photon intensity (180 μmol m^{-2} s^{-1}) were compared to those of blue light regimes of lower intensity (60 μmol m^{-2} s^{-1}), more mating occurred under red light in four of four trials, but none were significantly different. Mating pairs of three types were observed: oblong-oblong (o-o), oblong-sphere (o-s) and sphere-sphere (s-s). Cell packs that produced mating types and o-o mating pairs diminished with time. However, o-s and s-s mating pairs and quadriflagellate zygotes produced from the mating pairs increased with time.

KEY WORDS snow algae; *Chloromonas*; light intensity; light wavelength; mating

INTRODUCTION

Since the first report of snow algae in the literature (Bauer, 1819), these microbes have been found in snow world wide except for Africa (Kol, 1968; Hoham, 1980). Their presence in snow affects albedo and glacial melting. In the Sierra Nevada Mtns, California, red-colored snow algae enhanced solar absorption in snow on the order of 7-12% (Thomas and Duval, 1995), and snow algae on a Himalayan glacier accelerated glacial melting and affected the mass balance of the glacier by forming dark-colored areas reducing surface albedo (Kohshima *et al.*, 1993). Snow algae affect pH values in snow from CO_2 consumption during photosynthesis, and the relationship between pH and algae in snow depends on the metabolic state and phase of the snow algal life cycle (Hoham *et al.*, 1989; 1993). Some snow algae (*Chloromonas*) appear to be adapting to high acidity in eastern North American snow (Hoham and Mohn, 1985). Distributions of snow algae in snowpacks is spatial, and this may correlate with spatially distributed nutrients (Hoham, 1980; Tranter *et al.*, 1987; Davies *et al.*, 1989). Nutrient depletion caused by snow algae (particularly nitrogen) coincides with shifts in phases of the life cycle in the snow alga, *Chloromonas* (Hoham *et al.*, 1989; Jones, 1991).

Most snow algae belong to the green algae (Chlorophyta) and have swimming or flagellate stages in their life cycle. Active metabolic phases occur under conditions of snowmelt, available nutrients and dissolved gases, and penetration of light through the snowpack which is usually in spring or summer (Hoham, 1980). The process begins with germination of resting spores at the snow-soil interface [old snow - new snow interface in persistent snowfields] producing biflagellate zoospores (Hoham, 1980). These cells swim in the liquid meltwater surrounding the snow crystals towards the upper part of the snowpack, and their position in the snowpack is determined by light intensity and wavelength. Visible blooms of snow algae occur a few to several days after germination. Both asexual and sexual biflagellates develop in some species. The sexual cells (gametes) fuse to form resting zygotes, and in other species, asexual resting spores develop directly from asexual biflagellate vegetative cells (Hoham,

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1980; Hoham *et al.*, 1993; Ling, 1996). The resting spores eventually adhere to the soil or debris over the soil when the snowpack has melted or remain on old snow in persistent snowfields. From year to year, populations of snow algae stay in approximately the same localities. The resting spores remain dormant during summer, may form daughter cells through cell division after the first freezes in autumn, are covered with new snow in fall, winter and spring, and do not germinate again until the factors repeat themselves as given above (Hoham, 1980).

Chloromonas sp.-D, a snow alga isolated from the Tughill Plateau, New York State (Hoham *et al.*, 1993), was examined under laboratory conditions for the effects of light on gametogenesis (development of gametes) and mating (A species name will be given to *Chloromonas* sp.-D when its life cycle is more completely understood). Other species of *Chloromonas* from snow have been examined in the field for life cycle development (Hoham, 1975; Hoham and Mullet, 1977; Hoham *et al.*, 1979; 1983; Ling and Seppelt, 1993). In some algae such as diatoms, the level of irradiance was more important than spectral composition for growth rates (Gostan *et al.*, 1986; Morel *et al.*, 1987; Humbeck *et al.*, 1988). In one diatom, light intensity increased biomass to a point, but higher light intensities were inhibitory (Saavedra and Voltina, 1996), and the same results were reported for the green alga, *Chlorella* (Hirata *et al.*, 1996). In the closely related *Chlamydomonas*, a blue light signal is required for the conversion of pregametes to gametes (Treier *et al.*, 1989; Beck and Treier, 1991). In *Chlamydomonas*, blue light triggers phototactic responses that may increase mating by bringing cells closer together (Takahashi and Watanabe, 1993) and increases flagellar agglutinins (sticky glycoproteins) for mating (Quarmby, 1994). In this study of *Chloromonas* sp.-D, the following questions were asked: 1) Which strains (isolates) were mating compatible?, 2) What effects do light wavelength and intensity have on mating?, and 3) What is the relationship between mating and time?

STUDY SITE AND METHODS

Study site and isolates. All isolates for this study were collected from Whetstone Gulf State Park, NY, in April 1988 at elevation 380 m (Hoham *et al.*, 1993). The site slopes NE and the algae were collected near the base of hardwoods such as sugar maple. Other trees in the vicinity were eastern hemlock, American beech and alder. Samples were taken from meltwater in the upper 20 cm of snowpacks that were usually under 50 cm in depth using sterile Whirl-Pak bags that were inverted over a hand as a glove. This allowed for sterile sampling without human contamination. The six isolates (strains) used in this study were 581A, C and D and 582A, C and D.

Laboratory mating procedures. Mating procedures were modified from those of Hoshaw (1961) for green algae. Cells were grown on M-1 medium (Hoham *et al.*, 1979) minus nitrogen agar plates for a minimum of two weeks prior to mating. Plates were placed in a Percival model CTS-66 Growth Chamber at 4°C in a 16:8 hr, light:dark photoperiod under GE 7.5W/130V clear incandescent bulbs and GE F40PL/AQ Wide-Spectrum (WS) fluorescent lighting at a photon intensity between 100-250 μmol m^{-2} s^{-1}. Isolates were washed from agar plates after the two week period using sterile demineralized water and concentrations of the two strains to be crossed were equalized at 1.5 X 10^6 cells ml^{-1}. These equalized cell suspensions were placed back into the growth chamber under a premating photoperiod of 6:8 hr, light:dark prior to time zero. At time zero, 4 ml aliquots of each strain were mixed (8 ml total) in sterile test tubes and allowed to mate for 0.5, 2, 4, 6 and 8 hrs.

All test tubes were placed in holders constructed of white acrylic that were designed by R.C. Johnson. Controls included Wide-Spectrum (WS) or Cool-White (CW) fluorescent lighting, the standard type of lighting used for growing algae in the laboratory. Experimentals included holders wrapped with either blue, green or red cellophane placed under either WS or CW lighting. Light transmission peaks for each regime were established using an International Light, IL-700 Radiometer connected to an IL-760 Photomultiplier. Light intensity readings were recorded in photon units (μmol m^{-2} s^{-1}) using a Li-Cor LI-1935a, 3-D spherical sensor attached to a LI-1000 Datalogger. This spherical sensor was ideal because it closely resembled the shape of test tubes, and it was placed inside the acrylic test tubes holders (with or without cellophane) to measure light intensity. Similar or different light intensities were used in the experiments. All cellophanes were tested for transmission peaks at Li-Cor Co., Lincoln, Nebr., with a LI-1800-22 Portable Spectroradiometer using a LI-1800-12S integrating sphere.

Fixation, tabulations and statistics. At the end of each mating trial, cells were fixed for 0.5 hr with 4% OsO_4 (osmium tetroxide) solution by applying it to about 75% of a Whatman filter paper that was taped to the lid of a Petri plate. From the 8 ml mating solution, a 3 ml aliquot was placed in the bottom of the Petri plate, the

lid with osmium tetroxide was placed over the bottom plate and fixation began. All fixations were done on ice within a Rubbermaid container. The container was placed back into the growth chamber to avoid any temperature shock and was tilted by placing a pencil under it at one end to collect the algal cells in a concentrated pool for fixation. After 0.5 hr of fixation, cells were collected and placed into new test tubes for tabulation.

Prior to tabulating cells, test tubes were gently rolled on a counter top to distribute the cells evenly before making microscope slides. Slides were prepared using Pasteur pipettes and cells were observed using Zeiss phase-contrast microscopes. Using a line transect counting technique, a total of 400 cells were counted as recommended by Guillard (1975) for a standard 10% error. Tabulations included oblong flagellate and non-flagellate cells, spherical flagellate and non-flagellate cells, cell packs with 2, 4 or 8 daughter cells, quadriflagellate zygotes and matings. Matings involving the normal two cells included oblong-oblong (o-o), oblong-sphere (o-s) and sphere-sphere (s-s) configurations. Abnormal matings were uncommon, but did include triples (3 flagellate cells grouped together) and rarely other configurations.

Z-tests were used between experiments to compare proportions of total matings observed to the total number of observations made. Critical Z-values for $P<0.05$, $P<0.01$ and $P<0.001$ were 1.960, 2.576 and 3.291 respectively. Regression analyses were done to determine relationships between two variables and plots of residuals were produced to determine validity, as well as R^2 values. Analysis of variance (ANOVA) was used to analyze differences in matings through time and significance of regressions.

Photomicroscopy. A Zeiss standard RA research microscope equipped with a Hitachi Denshi KP-D50 color digital camera was used. Image processing was done on a Magitronic 486 DX50 8MB PC, with Image Pro Plus software version 1.3.1. Cells were viewed using either a 100X (oil) phase-contrast or Nomarski-interference contrast objective. Contrast and brightness settings in the photomicrographs were manipulated to maximize clarity, and stored images were retrieved when needed.

RESULTS

Mating strains. Of the six strains or isolates, 582C and 582D are normal cross-mating strains. Strains 581C and 581D are self-mating strains and do not cross with one another, but only with themselves. Strains 581A and 582A are mating incompatible; these strains do not mate with one another or with themselves. Strains 581A and 582A may cross with 582C or 582D, but this has not been tested.

The effects of light wavelength on mating. The transmission peaks for the five light regimes used in these experiments are shown in Figures 1-5. In the controls, WS peaks in the red end of the spectrum (640 nm; Figure 1), while CW peaks in green-yellow light (580 nm; Figure 2), but emits considerably more blue light than does

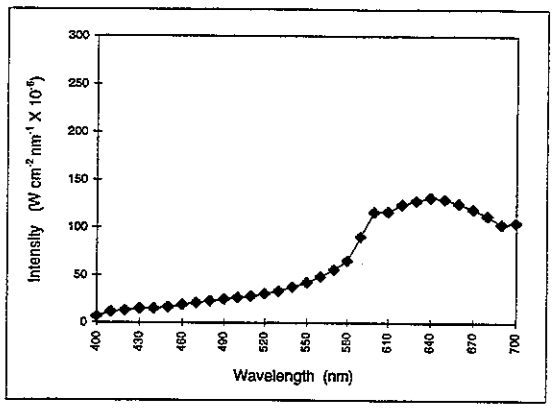


Figure 1. Transmission peak for Wide-Spectrum (WS)

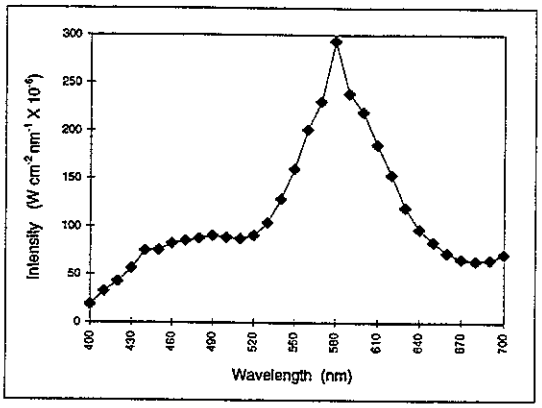


Figure 2. Transmission peak for Cool-White (CW)

WS. In the experimentals, red cellophane holders placed under WS peaks in red light (630 nm; Figure 3), blue cellophane holders placed under CW peaks in blue and green light (490 and 550 nm; Figure 4) and green cellophane holders placed under CW peaks in green-yellow light (580 nm; Figure 5). Red cellophane under WS

emits less blue and green light than WS alone, blue cellophane under CW emits more blue light than CW alone, and green cellophane under CW emits less blue light than CW alone.

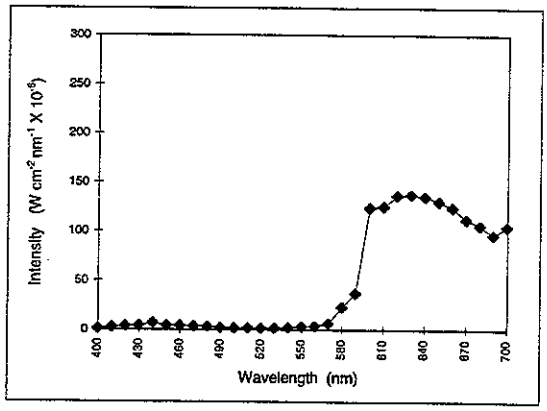


Figure 3. Transmission peak for red cellophane/WS

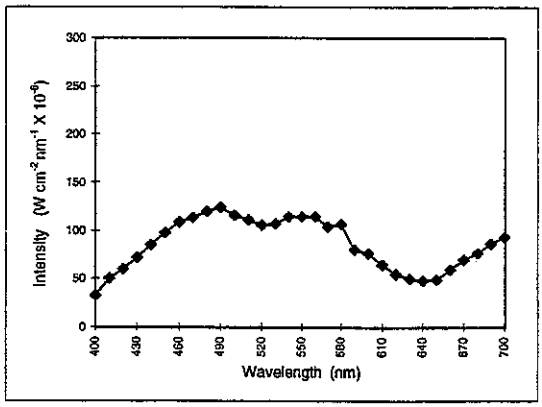


Figure 4. Transmission peak for blue cellophane/CW

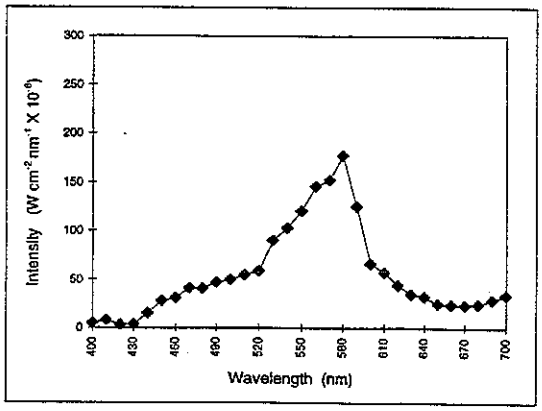


Figure 5. Transmission peak for green cellophane/CW

In four experiments where mating was compared in blue and green light regimes, more mating occurred under blue regimes in all four trials, and two of these were significantly different ($P<0.05$) (Table 1). In eight

Table 1. Summary of 4 experiments crossing *Chloromonas* sp.-D, strains 582C X 582D, comparing total matings between blue and green light regimes with similar light intensities.

Experiment	Light regime	Total matings[1]	Light intensity (μmol m^{-2} s^{-1})
1	CW green cellophane/CW	112 98	90 100
2	CW green cellophane/CW	151* 80*	90 100
3	blue cellophane/CW green cellophane/CW	112 98	100 100
4	blue cellophane/CW green cellophane/CW	129* 80*	100 100

[1]out of 2000 observations

*significant differences between light regimes ($P<0.05$)

experiments where mating was compared in blue light and red light regimes, more mating occurred under blue light in seven of eight trials, and three of these were significantly different ($P<0.05$) (Table 2). In three experiments comparing WS to red cellophane under WS, more mating occurred under WS in all three trials, and one was significantly different ($P<0.05$) (Table 3). When comparing total matings between red cellophane under WS and green cellophane under CW regimes, there was no trend (Table 4).

Table 2. Summary of 8 experiments crossing *Chloromonas* sp.-D, strains 582C X 582D, comparing total mating between blue and red light regimes with similar light intensities.

Experiment	Light regime	Total matings[1]	Light intensity (μmol m^{-2} s^{-1})
1	CW WS	112 90	90 90
2	CW WS	151 141	90 90
3	CW red cellophane/WS	112* 69*	100 100
4	CW red cellophane/WS	151* 70*	100 100
5	blue cellophane/CW red cellophane/WS	112* 69*	100 100
6	blue cellophane/CW red cellophane/WS	129 105	100 100
7	blue cellophane/CW WS	112 90	100 90
8	blue cellophane/CW WS	129 141	100 90

[1]out of 2000 observations
*significant differences between light regimes ($P<0.05$)

Table 3. Summary of 3 experiments crossing *Chloromonas* sp.-D, strains 582C X 582D, comparing total mating between red light regimes with similar light intensities (WS with more blue emission).

Experiment	Light regime	Total matings[1]	Light intensity (μmol m^{-2} s^{-1})
1	WS red cellophane/WS	82 72	180 180
2	WS red cellophane/WS	90 69	90 100
3	WS red cellophane/WS	141* 105*	90 100

[1]out of 2000 observations
*significant differences between light regimes ($P<0.05$)

The effects of light intensity on mating. More mating occurred under CW than under WS at all light (photon) intensities tested, and mating peaked at 200 μmol m^{-2} s^{-1} in both regimes (Figure 6). Lower light intensity produced more o-o mating pairs; however, higher light intensities produced more s-o and s-s mating pairs (Figure 7). When examining just the spherical-involved mating pairs (s-o and s-s), CW light regimes produced more of these mating pairs at lower light intensities and WS produced more at higher light intensities (Figure 8). When comparing red light regimes under 180 μmol m^{-2} s^{-1} to blue light regimes under 60 μmol m^{-2} s^{-1}, more mating

occurred under red light regimes in all four experiments, but none were significantly different (Table 5).

Table 4. Summary of 3 experiments crossing *Chloromonas* sp.-D, strains 582C X 582D, comparing total mating between green and red light regimes with similar light intensities.

Experiment	Light regime	Total matings[1]	Light intensity (μmol m^{-2} s^{-1})
1	green cellophane/CW red cellophane/WS	51 72	180 180
2	green cellophane/CW red cellophane/WS	98* 69*	100 100
3	green cellophane/CW red cellophane/WS	80 105	100 100

[1]out of 2000 observations
*significant differences between light regimes ($P<0.05$)

Table 5. Summary of 4 experiments crossing *Chloromonas* sp.-D, strains 582C X 582D, comparing total mating between blue and red light regimes with different light intensities.

Experiment	Light regime	Total matings[1]	Light intensity (μmol m^{-2} s^{-1})
1	CW WS	71 82	60 180
2	CW WS	71 72	60 180
3	blue cellophane/CW red cellophane/WS	64 72	60 180
4	blue cellophane/CW WS	64 82	60 180

[1]out of 2000 observations
*significant differences between light regimes ($P<0.05$)

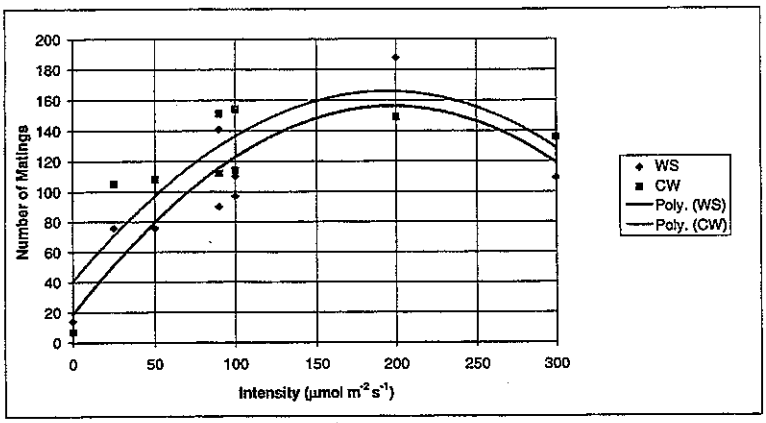


Figure 6. The relationship of photon intensity to total mating pairs for *Chloromonas* sp.-D using WS and CW light sources (WS: $y=-0.0035x^2+1.3906+18.814$, $R^2=0.7875$, $F=11.115$, $P<0.01$; CW: $y=-0.0033x^2+1.2876+40.839$, $R^2=0.73290$, $F=8.2302$, $P<0.05$)

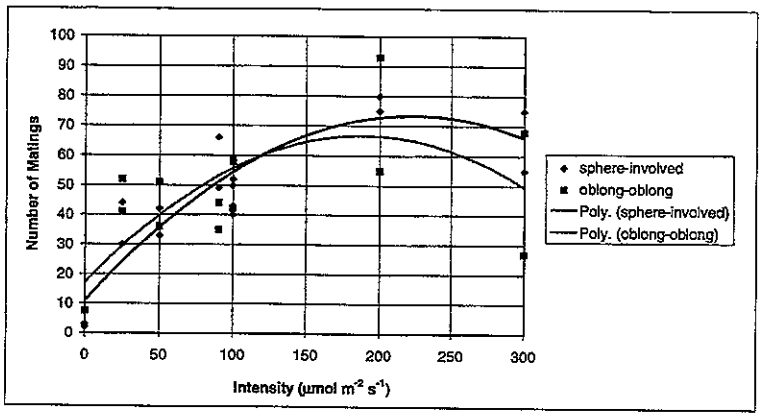


Figure 7. The relationship of photon intensity to o-o mating pairs and sphere-involved (o-s + s-s) mating pairs for *Chloromonas* sp.-D combining results from WS and CW light sources (o-o: $y=-0.0014x^2+0.5225+17.247$, $R^2=0.5264$, $F=7.1021$, $P<0.01$; s-involved: $y=-0.0013x^2+0.5600+10.918$, $R^2=0.8088$, $F=8.854$, $P<0.01$)

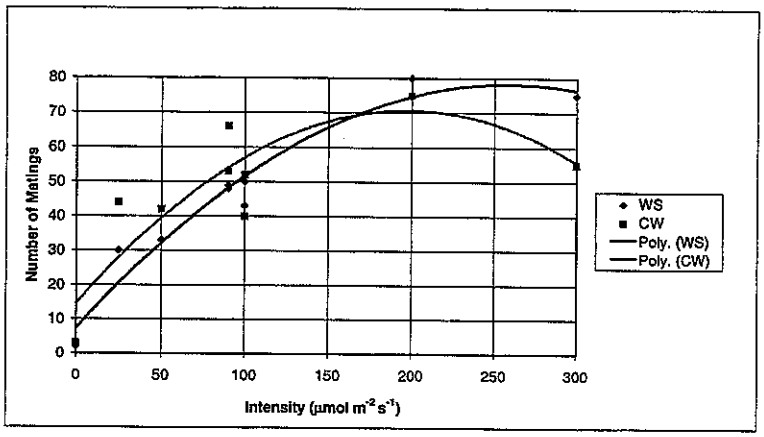


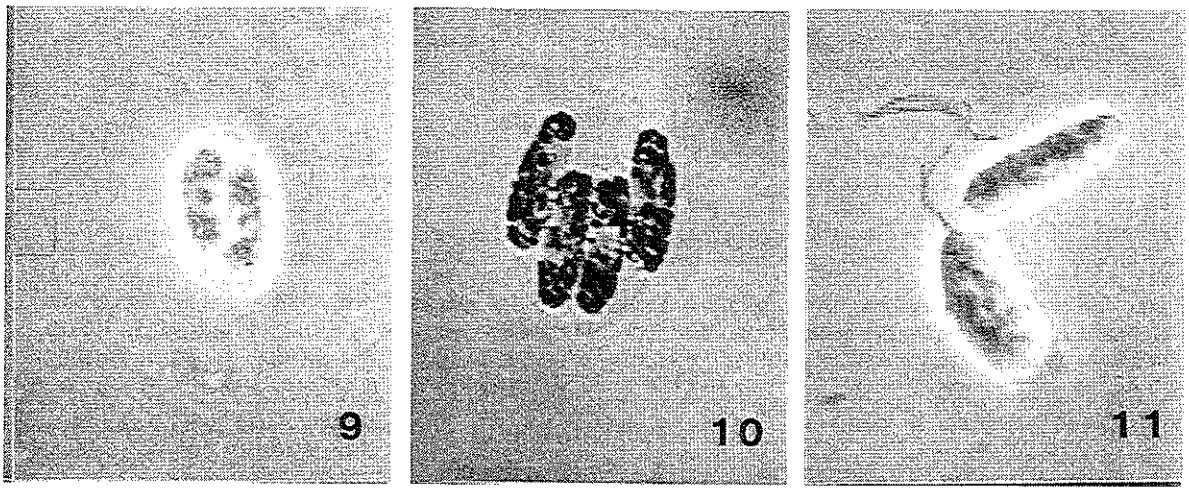
Figure 8. The relationship of photon intensity to sphere-involved mating pairs for *Chloromonas* sp.-D using WS and CW light sources (WS: $y=-0.0011x^2+0.5487+7.2878$, $R^2=0.9477$, $F=54.393$, $P<0.0001$; CW: $y=0.0014x^2+0.5677+14.543$, $R^2=0.7327$, $F=8.223$, $P<0.05$)

Life cycle development with time. From the 0.5-8 hour time trials used in the experiments, there were several correlations that were observed through time. The cell packs (Figures 9-10) that produced the gametes involved in mating declined with time. Total matings increased with time, but leveled off between hours 6-7. The three mating configurations differed with time, where o-o mating pairs (Figure 11) declined with time, both o-s (Figure 12) and s-s (Figure 13) mating pairs increased with time. Finally, the quadriflagellate zygotes (Figure 14) derived from the mating pairs increased with time.

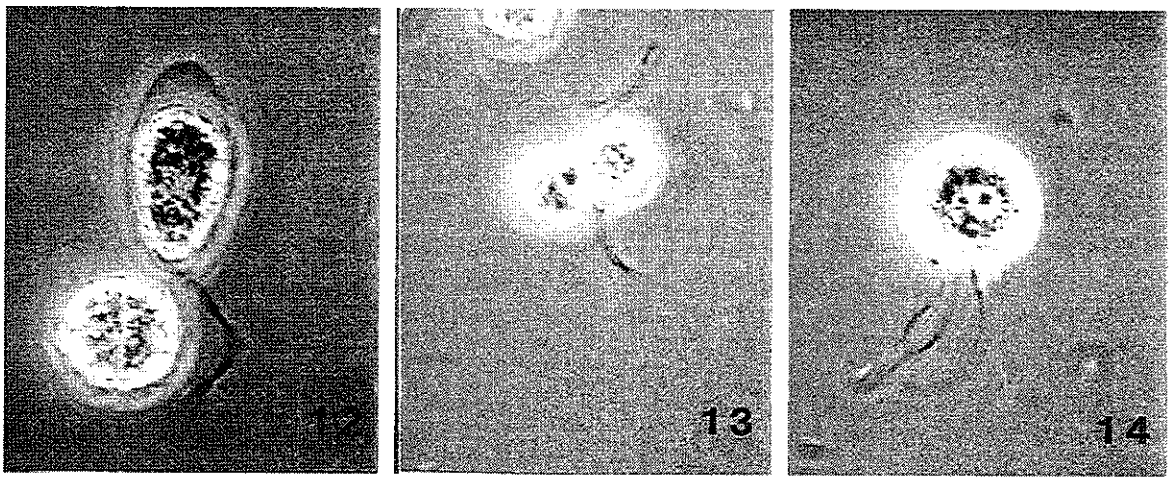
DISCUSSION

These light experiments were conducted using the normal mating strains, 582C and 582D, and blue light regimes increase the mating potential in this species of *Chloromonas* from snow. Blue and green light induced gamete formation in the closely related, *Chlamydomonas* (Buerkle *et al.*, 1993), but the experiments presented here suggest that blue light increases mating over that found in green light (Table 1). Red light reduced mating in *Chlamydomonas* (Azuara and Aparicio, 1984), and red light produces fewer matings when compared to blue light regimes for *Chloromonas* sp.-D (Tables 2 and 3). When comparing the red and green light regime experiments (Table 4), neither regime produced a trend in the mating process for *Chloromonas*. The results of our experiments

suggest that blue light induces more mating in *Chloromonas* and that green and red light reduce or inhibit mating. This is contrary to reports for *Chlamydomonas* that suggest blue and green light promote mating and red light reduces mating. Other species of *Chloromonas* from snow need to be tested to see if correlations between light wavelength and gamete production are similar or different.



Figures 9-11. Figure 9. A cell pack of 2 daughter cells. Figure 10. A cell pack of 4 daughter cells. Figure 11. An oblong-oblong (o-o) mating pair. Scale bar (----------) = 15 μm for all Figures



Figures 12-14. Figure 12. An oblong-spherical (o-s) mating pair. Figure 13. A spherical-spherical (s-s) mating pair. Figure 14. A quadriflagellate zygote. Scale bar (----------) = 15 μm for all Figures

Light photon intensity had a profound effect on mating in *Chloromonas* with most mating occurring at 200 μmol m^{-2} s^{-1} under both WS and CW. However, more mating always occurred under the CW regime which emits more blue light than WS (See Figures 1-2, 6). Increasing light intensity also produced more s-o and s-s mating pairs than o-o mating pairs (Figure 7). Light intensity affects morphological, cellular and physiological processes in algae (Falkowski, 1980; Cullen, 1990). Photoinhibition occurred at higher light intensities in the alga (diatom), *Chaetoceros* (Saavedra and Voltolina, 1986; Morel *et al.*, 1987). The light intensities used in the experiments presented here (0-300 μmol m^{-2} s^{-1}) would be found in the upper 20 cm of a snowpack during time of snowmelt. This information is based on light intensity readings from snowpacks taken in the field in μW cm^{-2} nm^{-1} (Hoham, 1975; Hoham *et al.*, 1983) and converted to μmol m^{-2} s^{-1} (S. Roemer, LiCor Instrument Co., person. comm.).

Light intensities involving different wavelengths affected the development of gametes in *Chloromonas* sp-D. Higher intensity red light regimes increased mating over low intensity blue light regimes (Table 4). Higher light intensities involving CW regimes that emit more blue light decreased s-o and s-s matings in CW regimes compared to WS regimes, but the reverse was true for lower light intensities (Figure 8). It is apparent that a complex

interaction between light intensity and wavelength in *Chloromonas* sp.-D exists with respect to life cycle development and gametogenesis. Possibly action spectra of certain pigments might be enhanced or subdued at high or low light intensities as suggested by Galland and Lipson (1985), but this would need verification in *Chloromonas* sp.-D.

Anisogamous sexual reproduction (fusion of gametes of different sizes) was observed in other species of *Chloromonas* from snow (Hoham and Mullet, 1977; Hoham *et al.*, 1979; 1983) and in *Chlamydomonas* (Wiese *et al.*, 1979). All of the *Chloromonas* studies in snow were from field material, and some of these species have isogamous sexual reproduction as well (fusion of gametes of the same size). It is not known in these species how light wavelength or intensity control gametogenesis. It was also observed in these field studies from snow that most gametic fusions occurred just before or within 2 hours after sunrise. Thus *Chloromonas* sp.-D may be very different from other snow algae having sexual reproduction peaking at 6-7 hours after the light cycle begins in the laboratory rather than just before or after sunrise. From our observations, fewer than 6% of oblong cells were involved in mating and o-o mating pairs declined through time. With the exception of the 0.5 hour mating time period, however, more than 50% of all spheres were involved in mating, and o-s and s-s mating pairs increased with time. The increase in spherical cells through time correlated with the decline in the cell packs that produced them. Quadriflagellate zygotes produced from the mating pairs also increased with time which would be expected. However, it is not understood which environmental factors control zygote maturation.

Nutrient starvation (nitrogen) promotes gametogenesis in *Chlamydomonas* (Sager and Grannick, 1954; Kates and Jones, 1964; Beck and Acker, 1992). All of the experiments in this study involved algal cells deprived of nitrogen for a minimum of two weeks. However, in a few experiments, nitrogen was deprived for up to 60 days prior to mating, and this may account for differences in total matings when repeating experiments that used the same light intensity or wavelength on different dates.

In summary, gametogenesis and mating is maximized in *Chloromonas* sp.-D when using blue light regimes compared to other regimes at equal light intensities. Mating also peaked at an intensity of 200 μmol m^{-2} s^{-1} using both WS and CW light sources. Increasing the light intensity in red light regimes three times that of blue light regimes (180 vs 60 μmol m^{-2} s^{-1}) reversed the mating process with more mating occurring under red light. Mating peaked at 6-7 hours after mating began, but o-o mating pairs declined through time while s-o and s-s mating pairs increased with time.

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