A REVISION OF *NITELLA CRISTATA* BRAUN (CHARACEAE) AND ITS ALLIES.

Part I. Experimental Taxonomy.

By T. C. Chambers, University of Sydney, and Mary B. Williams.*

(Plate xviii; three Text-figures.)

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Synopsis.

Clones of three forms of *Nitella cristata* Braun (Characeae) were grown under different light, temperature and day-length conditions. The resulting environmental variations indicate that relative length and form of fertile and sterile whorls are not reliable diagnostic characters for this species.

Introduction.

Few cryptogamic groups have been subjected to controlled environment experiments such as are now commonly used in the experimental taxonomy of higher plants. Members of the Characeae are ideal subjects for these experiments as they are easy to propagate clonally and the non-sterile aqueous conditions that they demand require only the control of temperature and light, and avoid the complex problems of humidity adjustment. Here, possibly for the first time, these methods are applied to a multicellular algal group, in an attempt to sort out some difficult forms which may be referred to as "the *cristata* group" of *Nitella*.

Braun (1852) described *Nitella cristata*, based on material collected by Charles Stuart from South Esk River, Tasmania, in 1848, and *N. cristata* var. *ambigua*, based on a specimen collected by Ferdinand von Mueller near Melbourne, Victoria, in 1852. The variety was distinguished by having the fertile whorls not very distinct from the sterile ones and the spores smaller and with a thinner crest. No measurements were given in either case.

Kuetzing (1857) studied duplicates of the specimens seen by Braun (from Sonder's herbarium instead of Mueller's) and published drawings which are of very little use diagnostically. In their world monograph, Braun and Nordstedt (1882) added some measurements to the descriptions, but cited specimens merely as "Tasmanian" and "Neu-Holland sö" (south-east), probably in reference to the types.

Meanwhile, Braun (1860) examined a specimen collected by Gunn from near Launceston, Tasmania, and published for it the locality data with the nomen nudum *N. diffusa*. When Braun and Nordstedt (1882) validly published the name, the specimen was cited merely as "Tasmanien", but presumably this referred to Gunn's specimen.

N. diffusa was differentiated from *N. cristata* chiefly by the fertile whorls not being congested into heads; since this was also diagnostic of *N. cristata* var. *ambigua*, and since the spore sizes of the three taxa overlap, it is difficult to see how they could have been distinguished at this time. The features of the three taxa, as then known, are set out in Table 1.

Nordstedt (1889) drew attention to the importance of spore-wall decoration in distinguishing species. From "Tasmanien originalex" he described the spore-wall of *N. diffusa* as granulate; from Charles Stuart's collection No. 753 he described the outer spore-wall of *N. cristata* as tuberculate. Williams (1959) has shown that the spore-wall of the type (Stuart 219) differs from that of Stuart 753. The spore-wall of *N. cristata* var. *ambigua* was not described and the type specimen has not been traced.

* Linnean Macleay Fellow of the Society in Botany, 1955-58.

Subsequently Nordstedt (1918) and Groves and Allen (1935) have ignored *N. cristata* var. *ambigua* while maintaining *N. cristata* and *N. diffusa*, which they distinguished primarily on the characteristics of the fertile whorls, together with spore size and spore-wall decoration. Groves and Allen commented on the difficulty of separating the two species.

TABLE 1.

Morphological Features of Nitella diffusa *and Two Varieties of* N. cristata, *as Known in 1889.*

	N. cristata var. *cristata* Braun, 1852.	*N. cristata* var. *ambigua* Braun, 1852.	*N. diffusa* Braun in Braun and Nordstedt, 1882.
Original specimen	Stuart 219. In Tasmania *ad flumen* South Esk River, 1848. LECTOTYPE.	F. Mueller, Sept., 1882. "In aquis stagnantibus prope Melbourne."	Tasmania, rare, Gunn, 1574, Habitat, Distillery Creek. Launceston. Rivulet near Penquite. HOLOTYPE.
Stem diameter ..	Strong, to 1 mm.	0·72–0·75	0·5–0·6
Sterile branchlets ..	6	—	6
(*b*) Furcation ..	Simple or 1-furcate.	—	2×, partly 3×.
(*c*) Secondary rays	3–5	—	Not given.
(*d*) Tertiary rays ..	2–3	—	Not given.
(*e*) Dactyls ..	Very short, often forming 3-celled "mucro".	—	3–4 cells, gradually shorter and thinner, ultimate one very short and acute.
Fertile branchlets	Short, forming elongate, interrupted heads; twice furcate, dactyls rigid, diverging, 3-celled, gradually narrowing, acute.	Not sharply distinct from sterile.	Not sharply distinct from sterile. 5–6 mm. long. Forming heads (*fide* Willis).
Fertile whorls ..	Forming heads.	Not forming heads.	Not forming heads.
Spirals of oogonium	6–7	—	8
Oospore length ..	0·34–0·37	0·30–0·35	0·32–0·37
Spore width ..	0·31–0·32	0·26–0·28	0·30–0·32
Spore striae ..	Thick, prominent.	—	—
Spore wall ..	Sparsely verrucate outer, finely baculate inner.	Unknown.	Fine-grained dotted, i.e., finely baculate.
Antheridium ..	0·50–0·58 mm.	—	—

The following experiments were designed to test whether, in fact, the relative length and form of fertile whorls are modified by environment and therefore may be unreliable characters for taxonomic use. In addition it was hoped to gain some insight into the physiology of growth of *Nitella*.

EXPERIMENTAL MATERIAL.

It was not possible to examine experimentally a complete range of forms referable to *N. cristata*. The following were selected for experimental study, the choice being limited by the necessity of using the same sex in all experiments and of selecting forms representative of the morphological range. Voucher specimens have been prepared and will be lodged in the National Herbarium of New South Wales.

1. *Gledswood*.—A form with rather diffuse fertile whorls, referable to *N. cristata* var. *ambigua*.

Locality: A dam on "Gledswood" property 7 miles N.E. of Camden on the Hume Highway, N.S.W. *Voucher specimens*: 13.vii.1955, Mary B. Williams. 271.1, 271.2 (male and female).

TABLE 2.

Morphological Features of Three Forms Assigned to Nitella cristata *and Used in Controlled Environment Experiments.*

	Gledswood.	Kiefer's Dam.	Long Plains Creek.
Height (cm.)	10–15	5–10	15
Stem diameter (mm.) ..	0·5	0·5–0·575	0·75–1·0
Sterile branchlet number	6	6	6
Stem branchlet length (mm.)	Lower, 3–4 Upper, 1	Lower, 10 Upper, 6–8	Lower, 17 Upper, 10
Furcation	1–2 ×	1–2 ×	1 ×, occasionally partly 2 ×
Relative length primary ray.	$\frac{1}{2}$–$\frac{1}{3}$ total length.	$\frac{1}{2}$–$\frac{2}{3}$ total length, 3–4 mm.	Almost whole length exceeds $\frac{2}{3}$.
Secondary rays: Number .. .. Length (mm.) ..	 (2)–3–(5) 10–15	 (3)–4(5) 1–2·5	 (1–2)–3 Less than 1.
Tertiary rays: Number .. .. Length (mm.) ..	 2–3 1–1·8	 2–3 1	 — If present, less than 1.
Dactyl morphology ..	2–3 celled, lower cells gradually narrowing distally. Ultimate cell short, conical, acute.	2–3 celled, lower cells narrowing distally. Ultimate cell short, conical, acute.	2–3 celled, lower cells narrowing distally. Ultimate cell small, conical, acute.
Fertile branchlet length	Up to 5 mm.	2·5–5	Less than 2*
Fertile whorls	Lower fertile whorls similar to sterile, upper forming loose heads.	Lower fertile whorls like sterile, upper forming loose heads.	Sharply distinct from sterile.*
Oogonial position ..	Solitary or aggregate, generally at all free nodes.	Solitary or aggregate, generally at all free nodes.	Solitary at 1st or 2nd nodes.*
Oogonium length (mm.)	0·44–0·48	0·42–0·48	0·65
Spirals	7	—	—
Oospore length (mm.) ..	0·32–0·33	Unripe.	0·36–0·44*
Striae	5–6	—	5–6*
Spore wall†	Outer verrucate over inner finely baculate.	Outer verrucate over inner baculate.	Outer verrucate over inner baculate.
Antheridia length (mm.)	0·40–0·50	—	—
Chromosome number ..	9	? 9	? 9*

* Field material not fertile. Measurements taken from similar material collected Leech Creek, on Monaro Highway, 3 miles east of Nimmitabel, MBW No. 108.

† For terminology, see Williams, 1959.

2. *Kiefer's Dam*.—A form with compact fertile whorls, referable to *N. cristata* var. *ambigua*, but differing slightly from Gledswood.

Locality: A dam on the property of J. Kiefer, 3 miles N.E. of Armidale on Rockvale Road, N.S.W. *Voucher specimens*: 22.ix.1955, leg. J. A. Sutherland, Mary B. Williams 335; do., 28.x.1955, Mary B. Williams 333 (ex culture).

3. *Long Plains Creek*.—A form with restricted forking, very short dactyls and very compact fertile whorls, doubtfully referable to *N. cristata* Braun.

Locality: Long Plains Creek, on the old Adaminaby-Kiandra Road 5 miles from Adaminaby, N.S.W. *Voucher specimens*: 8.ii.1955, Mary B. Williams 106, 111; do., 24.xi.1955, Mary B. Williams 362 (ex culture). Also see a specimen from a creek on Monaro Highway, 3 miles E. of Nimmitabel, N.S.W., 7.ii.1955, Mary B. Williams 108.

Morphological features of original material of these three forms are compared in Table 2.

Methods.

Single male plants of each of the forms described in Table 2 were grown in enamel pots 17 cm. high holding 1 in. of sieved pond-mud covered with a few millimetres of sand and 4 litres of tap water. These were covered with a semi-opaque polythene film to reduce water loss and to diffuse light. After two months' growth in a glasshouse

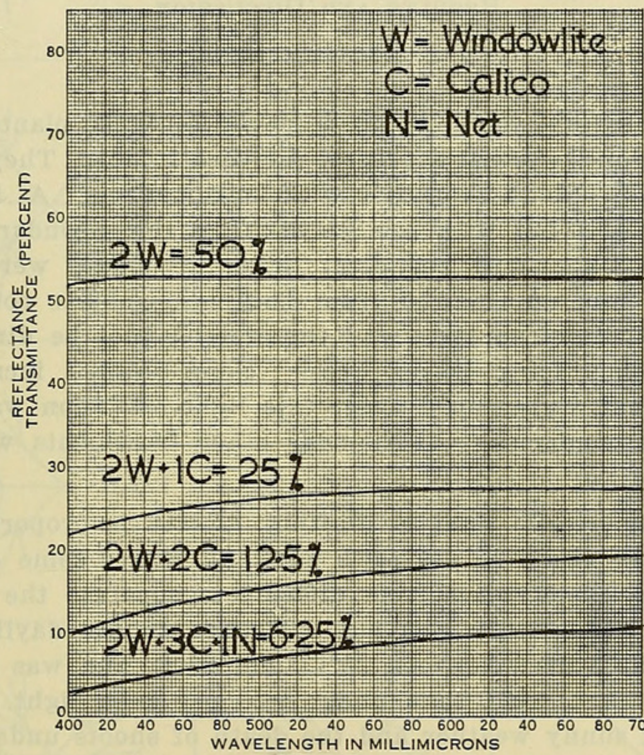

Fig. 1.—The light transmission characteristics of the "windowlite" screens.

(November-December, 1957), the resulting clones were dissected into shoot tip cuttings each three internodes long, and also into pieces of rooted bulbil. These ramets were planted singly in jars 7·5 cm. high holding 130 c.c. of mud covered with a few millimetres of sand. The mud was freshly collected from "Gledswood" pond and thoroughly mixed until homogeneous. The jars were placed in white enamel pots (15 in. high with a volume of 8 litres), 11 jars in each pot arranged so that there were 3 or 4 replicates of each of the 3 forms per pot. These pots were then filled with tapwater and placed in duplicate under each of the various temperature and light conditions described in Figures 2 and 3. The water was drained and replaced every second week.

The 6·25–100 per cent. light set were placed on open ground in full sunlight, or under screens made of one or more thicknesses of "windowlite" interleaved with calico and mosquito net, of which the transmission characteristics are described in Figure 1. These screens lacked any serious peaks of absorption and the light conditions should have been essentially those encountered by the field material. As the experiment was set up in December the plants in this set were subjected to long day conditions. The 15 degrees C. at 50 per cent light group were placed in a large water bath thermostatically controlled by refrigeration, and covered by a 50 per cent. light screen all within a glasshouse. The short-day set were placed in a thermostatically controlled room at 15 degrees C., and given 8 hours of light in 24 under thirteen 40-watt Osram "Warm White" tubes spaced at 1⅝ inches. The top of each pot was 7½ inches below the lights and a 50 per cent. light screen was placed on top of half the set.

At one month and again at two and a half months the clones were removed, sorted, and a typical strand selected from each plant. This was mounted in water (boiled, to minimize bubble formation) between glass plates in the negative carrier of an enlarger, and a direct photographic print of known magnification made. From these and with further reference to a preserved sample a series of average mature sterile whorl diagrams was constructed.

The percentage daylight, the low temperature at 50 per cent. daylight, and the short-day sets cannot be compared directly owing to the different qualities of daylight and fluorescent light.

Results and Discussion.

Daylight Intensity Experiment.

(*a*) *Vegetative Development.*

With increasing daylight intensity up to 25 per cent. the plants of the "Gledswood" and "Kiefer's Dam" clones showed a general increase in size. They had longer primary rays and larger whorls (Fig. 2) together with longer shoots. At 50 per cent. light the plants were more compact but with less growth, and the secondary rays were of more regular dimensions. Under 100 per cent. light the plants were distinctly stunted. However, this group was unprotected from both atmospheric contamination and the overheating effects of direct sunlight and therefore cannot be directly compared with other treatments. The "Long Plains Creek" clone showed increase in size of the rays up to 12·5 per cent. light; only under this light condition was there a noticeable development of the secondaries, which under other treatments were reduced to very small dimensions.

It is suggested that while light is limiting, growth is proportional to increase in intensity to somewhere above 25 per cent. daylight, where some other factor becomes limiting. With these experiments it is difficult to separate the effects of light and temperature because temperature was not controlled in the daylight set. The water temperature at midday varied between 25°C. and 29°C. and was usually 1°C. or 2°C. higher under 50 per cent. light than under 6·25 per cent. light. Plants in the field die down in very hot sunny weather and the death of shoots under these unfavourable "light" conditions may be related to increase in respiration dependent on the associated higher temperature. This has been shown to apply to *Chara australis* (Chambers, 1958; unpublished).

(*b*) *Reproductive Development.*

Sex organs were formed in the Kiefer's Dam and Gledswood clones under all conditions except 100 per cent. daylight, where the plants were not healthy. None of the Long Plains Creek plants formed sex organs under any of the conditions. The development of fertile whorls was first noted in 50 per cent. light after four weeks and occurred a little earlier in the Kiefer's Dam than in the Gledswood clone. The form of the fertile whorls was more diffuse in lower light (Pl. xviii).

Although it is possible that these plants with diffuse fruiting heads might ultimately have developed the compact habit of the higher light forms, nevertheless Plate xviii clearly illustrates that at a given age, mature plants of genetically identical clones may exhibit a range from diffuse to compact fertile whorls under the different light conditions. The shortening of whorl branchlets and internode length in high light intensity is perhaps analogous to rosette formation in higher plants.

This experiment shows that the length of fertile whorls relative to sterile is not a reliable diagnostic character for the determination of species.

Day-length Experiment.

In short-day conditions (8 hours' light) under fluorescent light the various clones under the 50 per cent. light screens were markedly larger and more diffuse than those in 100 per cent. light. The Gledswood and Kiefer's Dam plants diverged more noticeably than in the daylight experiment—the Gledswood having more forking and greater

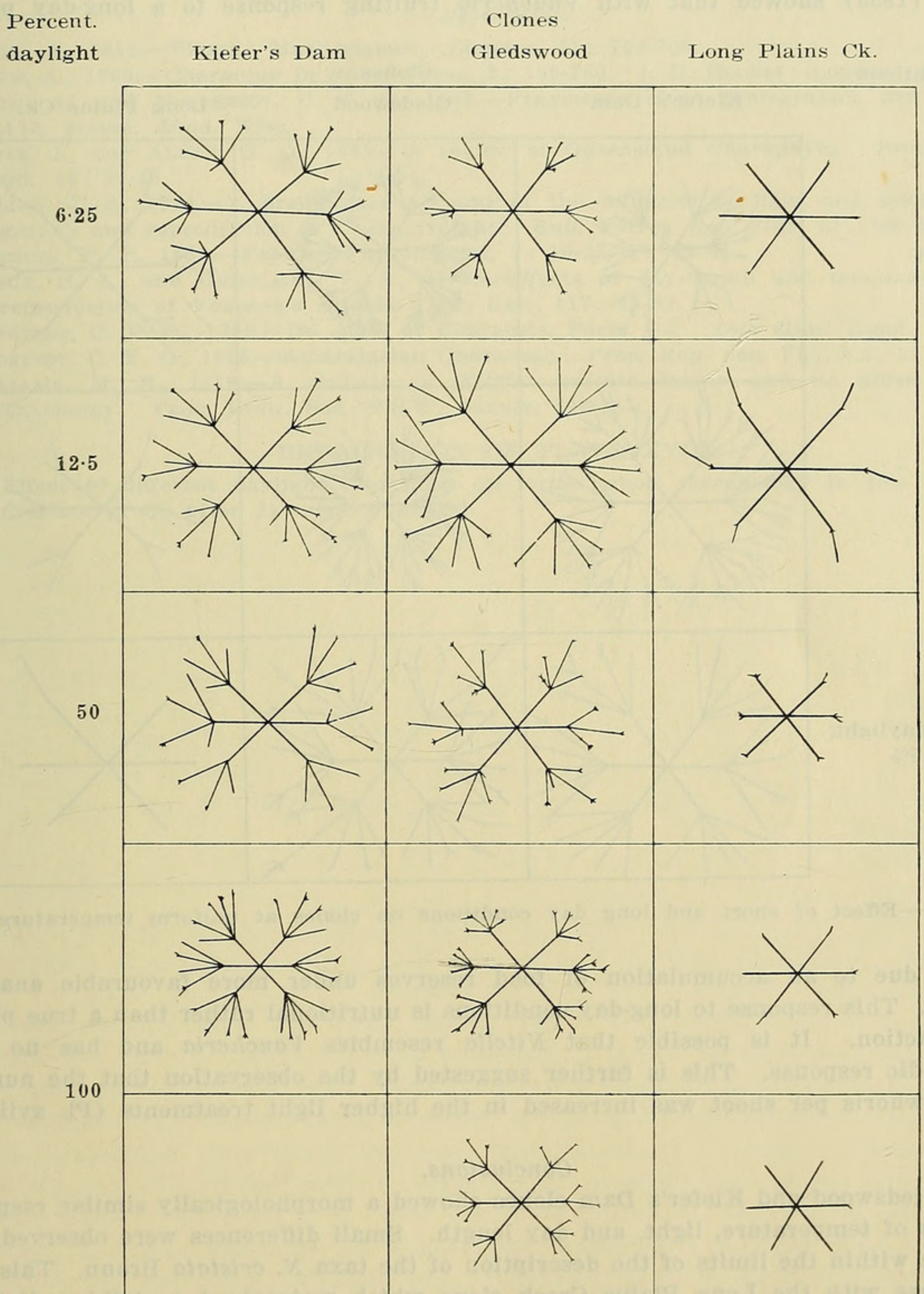

FIG. 2.—Effect of different daylight intensities on the morphology of the sterile whorls in three members of the *cristata* group after 2 months' growth.

development of tertiary and quaternary rays (Fig. 3). As this experiment was at 15°C. it is difficult to designate this as a light or temperature effect. However, plants kept at 15°C. long-day (30 per cent. daylight), although not directly comparable with other conditions, do show, in the low temperature, whorls much larger and more diffuse than in any of the other daylight conditions.

The Gledswood and Kiefer's Dam clones developed sex organs after one month's growth in both the short-day and long-day experimental conditions, the fertile whorls being longer in low light intensity as before. Formation of fertile whorls in short-day conditions could have been the result of pre-conditioning since the plants were grown from cuttings previously subjected to the long days of December. The photoperiod behaviour of *Nitella* is uncertain despite the work of Karling (1924). League and Greulach (1955) showed that with *Vaucheria* fruiting response to a long-day photo-

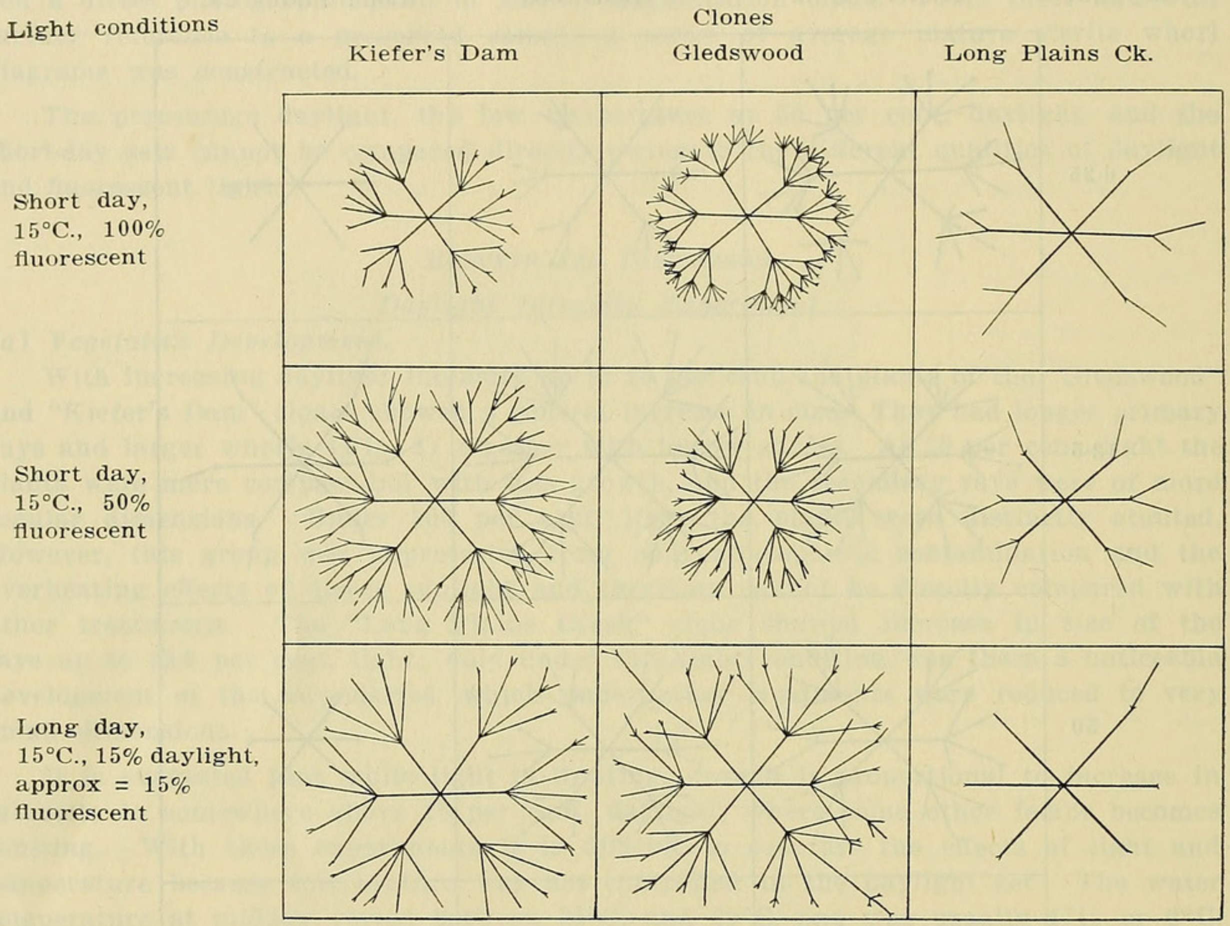

FIG. 3.—Effect of short and long day conditions on clones at uniform temperature.

period is due to an accumulation of food reserves under more favourable anabolic conditions. This response to long-day conditions is nutritional rather than a true photoperiod reaction. It is possible that *Nitella* resembles *Vaucheria* and has no true photoperiodic response. This is further suggested by the observation that the number of fertile whorls per shoot was increased in the higher light treatments (Pl. xviii).

Conclusions.

The Gledswood and Kiefer's Dam clones showed a morphologically similar response to changes of temperature, light, and day length. Small differences were observed, but these were within the limits of the description of the taxa *N. cristata* Braun. This was not the case with the Long Plains Creek clone which maintained a striking distinctness of form and fruiting behaviour which clearly suggests that it should be regarded as a separate species.

The taxonomic implications from the work described in this paper are used in a new treatment of the *Nitella cristata* group (Williams, 1959).

These findings demonstrate that techniques recognized as valuable in higher plant taxonomy are also useful when applied to lower groups, and an experimental approach to the variability of other difficult species in the Characeae would be of great benefit in clarifying their taxonomy.

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EXPLANATION OF PLATE XVIII.

Effect of different daylight intensities on fertile whorl morphology in two members of the *Cristata* group after 2½ months' growth.