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monosomes reported here contribute substantially to the ultimate goal of the establishment of monosomes for the 26 chromosomes of cotton. Other monosomes isolated recently are in the process of being identified by both cytologic and genetic methods.

Monosomic lines provide a means of replacing any one chromosome by its homologue in another variety or breeding stock and evaluating it genetically. Work of this type is in progress with the chromosomes that have been identified by monosomic analysis.

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## A POPULATION STUDY OF CHARA ZEYLANICA IN TEXAS, OKLAHOMA, AND NEW MEXICO<sup>1</sup>

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## ABSTRACT

Examination of 140 collections of *Chara zeylanica* from 3 southwestern states shows that a strong dichotomy exists in the collections. Some produce 4-plated antheridia while others produce 8-plated antheridia. Absolute separation of the study material is possible on the basis of plate number. In addition, 3 other characters (length/width ratio of oospores; fertility of first branchlet node; and number of distal ecorticate branchlet internodes) show strong correlation with the antheridial type. Preliminary chromosomal work indicates that a segregation at this level may also be possible. In 4-plated collections examined, n=28; 8-plated collections are usually n=56; however, a few 8-plated collections are n=42. No conclusion has been reached regarding the status of the latter collections.

WITHIN the Division Charophyta are several polymorphic species. *Chara zeylanica* Klein ex Willd. is representative of those elements showing broad limits of variability. In the literature (Robinson, 1906; Horn af Rantzien, 1950; Zaneveld, 1940; Daily, 1953) importance has been given to such characters as fertility of the first branchlet node, "spininess," number of branchlet internodes (corticated and ecorticated),

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and size of oospores in describing subspecific taxa for this species. The present paper considers still another character in treating the species, that of the number of antheridial shield-cells. Sundaralingam (1960) expressed doubt as to the constancy of this character, and hence, as to its usefulness at the species level. The authors, however, have found that among their collections the number of antheridial shield-cells is quite constant. It is felt that within the *C. zeylanica* complex this character should be given greater importance than has been the case in the past.

Groves (1931) pointed out that the antheridia of C. zeylanica were 4-plated, producing 4 lozengeshaped shield-cells instead of 8 triangular ones, as is the case for all other species in the genus

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(Sundaralingam, 1959). Sundaralingam (1954) mentioned that the antheridia of this species were 8-plated. Later, Sundaralingam and Francis (1958) and Sundaralingam (1959) stated that C. zeylanica produced 4-plated antheridia. It is evident from these papers that the authors were either not aware that they had on 3 separate occasions published 2 different plate numbers in the species, or, being aware of it, attributed no im-portance to such a difference. In any case, no one has specifically pointed out in the same paper that in C. zeylanica both antheridial types occur. As far as is known, the plants breed true, 4-plated collections never giving rise to 8-plated progeny and vice versa. The questions are then raised as to the usefulness of the antheridial plate number as a character at the specific or infraspecific level and whether hybridization might not occur.

METHODS AND MATERIALS—Some 140 field collections of *C. zeylanica* from Texas, Oklahoma, and New Mexico were transplanted to widemouth 1-gal jars to which a small amount of garden soil and tap water had been added. These cultures were maintained in the greenhouse for the duration of the study. From the greenhouse for the duration of the study. From the greenhouse cultures, various vegetative and reproductive data were gathered. Voucher specimens are on deposit at Texas Technological College, Lubbock, and Butler University, Indianapolis.

Antheridia—Each collection was recorded as producing either 4- or 8-plated antheridia (Fig. 1, 2). Because this was the strongest character on which to separate the collections (100% separability), all other vegetative and reproductive characters were analyzed in terms of their segregation along the line of antheridial type.

Branchlets—To analyze the difference between 4- and 8-plated collections with respect to various branchlet characters, 9 branchlets were selected from each collection. Three branchlets were excised from the main axis of 3 separate plants. The branchlets were taken from 3 successive whorls, the uppermost fertile whorl (i.e., producing ripe oospores) and the whorls immediately above and below that whorl. Data were gathered for 2 characters, namely, fertility of the first (lowermost) branchlet node and the number of distal ecorticate branchlet internodes.

Oospores-One-hundred oospores from each of 29 collections were chosen for an analysis of the differences in mean length, width, and length/ width ratio. These collections were made in the field where ample amounts of plant material were gathered into rinse pans and vigorously kneaded. The separated masses of oospores were then dried. As the amount of lime deposition both inside and outside the enveloping cells of the oogonium is probably not entirely a matter of the plant's genotype, it was felt that this lime covering should be removed and only the outer dimensions of the original egg cell be measured. This was accomplished initially by gently crushing the lime shell with a dowel; later it was discovered that the lime could be removed just as effectively by soaking the oospores in a 5% solution of acetic acid for 6-8 hr. This technique did not alter the size of the oospores and did permit accurate measurements. For the range of variation in length/



Fig. 1–2.—Fig. 1. Photomicrograph of a shield-cell from a 4-plated antheridium of *Chara zeylanica*,  $\times 100$ .—Fig. 2. Photomicrograph of 4 shield-cells from an 8-plated antheridium of *Chara zeylanica*,  $\times 100$ .

	Ν	Range of Xs L/W ratios	Range of S.D.s of Xs	Range of X lengths $(\mu)$	Range of $\overline{X}$ widths $(\mu)$
4-plated Collections	1500	1.90-2.69	0.084-0.228	503-677	211-312
8-plated Collections	1400	1.39 - 1.60	0.024 – 0.097	567 - 749	392 - 514

TABLE 1. Statistical data<sup>a</sup> from oospore analysis of Chara zeylanica

 $^{a}$  N = number of observations; X = mean; S.D. = standard deviation.

width ratio from each collection, a mean, standard deviation, and standard error were calculated.

RESULTS—In Texas, Oklahoma, and New Mexico, C. zeylanica occurs as either a 4- or 8-plated plant with respect to antheridial shieldcell number. These 2 "types" are generally not found growing together; however, 3 locations were visited where both were fully intermixed in the same pond or tank (cf. collections 31–506; 493–494; 496–497, Fig. 3, 4). In each of the 3 locations, the same differences noted for geographically allopatric collections, especially with regard to oosporal characteristics, were maintained.

Antheridia—Detailed observation of the antheridia of C. zeylanica with respect to the number and shape of individual shield-cells confirms the figures and statements of Groves (1931) and Sundaralingam and Francis (1958) that shieldcells from 8-plated antheridia are triangular while those from 4-plated antheridia are lozenge-shaped.



Fig. 3. Map of study area indicating locations of collections of *Chara zeylanica* used in oospore analysis. Map symbols are:  $\blacktriangle$  4-plated collections;  $\bigcirc$  8-plated collections;  $\bigcirc$  8-plated collections taken from same pond.

Collections within the study area show absolute separability on the basis of this character.

Oospores—Examination of 1500 oospores from 15 four-plated collections (number of observations = 100 for each collection) and 1400 from 14 eight-plated collections shows that the 2 types are separable within 2 standard deviations of the means on the basis of length/width ratio (Fig. 4 and Table 1). It is seen that the 4-plated collections are consistently more variable on the basis of this character than are 8-plated collections. Within certain limits, the plasticity of gene expression, at least when considering length/width ratio, appears to be characteristic of the species. Data (in preparation) gathered for several other species of Chara bear this out. Whether oospore size has adaptive value or not is not clear at present.

Fertility of first branchlet node—This character has been used to differentiate local forms of C. zeylanica (Zaneveld, 1940; Daily, 1953; Sundaralingam, 1959). It also proved to be a character of strong segregation among the study collections. Of 59 four-plated collections, 93%produced gametangia at the first node while 7%did not. Among 71 eight-plated collections, 7%produced gametangia at the first node but 86%did not.

Number of distal ecorticate branchlet internodes— As in the case of the length/width ratio of oospores, the amount of distal ecortication on the branchlets showed greater variability among 4-plated collections than among 8-plated ones. The range of variation for this character among 4-plated collections was 0–9 (number of observations = 477) with a mean value of 3. Among the 8-plated collections the range of variation was 0–3 (number of observations = 531) with a mean value of 0.2, the mode being 0.

Chromosome counts—Although some chromosome counts in the Charophyta have been reported (Hotchkiss, 1958; Gillet, 1959), a great deal more work must be done before any broad conclusions can be reached. In the present work, 9 collections of *C. zeylanica* were used in making chromosome counts from antheridial filament

Fig. 4. Variation in length/width ratio of oospores for *Chara zeylanica*. Horizontal lines show ranges of variation; vertical lines show mean ratio; open boxes represent 2 standard deviations on each side of the mean; closed boxes represent 2 standard deviations on each side of the mean; antheridial plate number to the right. N = 100 for each collection



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cells. Four 4-plated collections (257, 493, 509, and X-008) were examined, all of which had a haploid number of 28. Counts were made on 5 eight-plated collections, 3 of which (406, 494, and X-003) had a haploid number of 56, and 2 of which (508 and X-004) had a haploid number of 42. Material from which these counts were made is on deposit with Dr. Arland Hotchkiss at the University of Louisville, Louisville, Kentucky.

CONCLUSIONS—From the data presented here, it is concluded that 4- and 8-plated populations of *C. zeylanica* represent entities within the "*zeylanica*" complex between which little or no gene flow occurs. Further work is needed to determine the exact position of the n = 42 plants, whether they represent true hybrids between the populations having chromosome numbers of 28 and 56.

Although no one has yet attempted to rank the number of antheridial shield-cells with other morphological characters, the apparent truebreeding nature of the 2 "types" would require that this character be seriously considered when any "finalized" work is contemplated dealing with this section of the genus.

The degree of separability of the collections on the basis of oospore length/width ratio is also considered to be significant. A survey of published data indicates that, in several other species of phylogenetic proximity, oospore size shows as great or less of a difference as cited here for 4- and 8-plated collections of *C. zeylanica*.

In addition, a strong segregation of the collections is possible on the basis of fertility of the first branchlet node and the number of distal ecorticate branchlet internodes.

Chromosomal evidence is as yet inconclusive. Counts reported here plus subsequent investigations show that 4-plated collections are uniformly n = 28. Eight-plated collections, while mainly having an n = 56 number, may also occasionally be found with n = 42. There is every indication (personal communication from Dr. Hotchkiss) that other numbers occasionally occur in the species; however, whether they are 4- or 8-plated plants is not known at present.

Destruction of the type during the war requires that a revisit to the type locality (Malabar coast, India) be made. The authors feel that this should be done before any proposal of a new name is considered. Also, collections from other parts of the range will need to be examined before any change in this species' status enters the literature.

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