REPRODUCTIVE ISOLATION BETWEEN CYTOTYPES OF *CHARA CONTRARIA*

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INTRODUCTION

Few topics in evolutionary biology have generated as much discussion as have attempts to delimit species. Despite a certain amount of dissent, the genetically defined "biological species" of Dobzhansky (1941), Stebbins (1950), Mayr (1963, 1969), and others has gained wide acceptance, particularly among zoologists. Central to this concept is the assumed cohesion of a common gene pool. Ehrlich and Raven (1969) have recently emphasized the role of selection in maintaining the identity of the species as opposed to cohesion of the species gene pool. They have suggested that the unit of evolution may not necessarily coincide with the "biological species." This study continues the attempt to clarify the nature of the evolutionary unit in the genus Chara and thus constitutes a test of the Ehrlich-Raven suggestion. Chara contraria was the organism chosen for this study because of its world wide distribution, frequent local occurrence, and experimental tractability.

Many "species complexes" in the genus Chara exhibit a series of apparent polyploid cytotypes (Hotchkiss 1958; Sarma and Kahn 1965, 1967; Guerlesquin 1967; Proctor 1970; Proctor and Wiman 1970). The taxonomic significance of these various chromosome races is

currently disputed. Sarma and Kahn (1965a, 1965b, 1967) suggest that chromosome number constitutes an important diagnostic character while Wood (1965) did not extensively use such information as a diagnostic character in his recent monograph. Presently, little or no experimental support for either position exists, although the need for such data is clear.

Recently, Proctor et al. 1967, Proctor 1970, Proctor and Wiman 1970, Proctor et al. 1970 have experimentally investigated breeding patterns of several cosmopolitan "species complexes." Such studies have been partially directed toward identification and evaluation of the role of spatial separation in the evolution of the group. These workers have been cognizant of the existence of different cytotypes but have not attempted to evaluate their significance specifically. The primary point of emphasis has been the examination of long range crossing patterns. Less is known about such breeding patterns in local populations.

The organism of choice, C. contraria, typifies much of the genus in that it has all of the following characteristics which--in combination--make it unique among the green algae and well suited to experimental biosystematic investigations: 1) a large, morphologically complex thallus; 2) gametangia which can be physically

emasculated for experimental breeding purposes; 3) large, easily counted chromosomes; 4) facultative outcrossing-inbreeding capabilities; 5) a world wide distribution and 6) easily met culture requirements.

This study seeks to exploit such characteristics to: 1) define distributional patterns of the chromosome races found to occur in C. contraria, 2) evaluate the taxonomic significance of the cytotypes, 3) elucidate breeding patterns extant in C. contraria populations with primary emphasis at the local level, and 4) interpret the results with the view of clarifying the nature of the evolutionary unit in the genus.

TAXONOMIC BACKGROUND

C. contraria Braun ex Kützing was first recognized as a specific entity in 1835. As Kützing (1845) published the first description of the plant, he, alone, is often cited as the nomenclatural authority (cf. Groves and Bullock-Webster 1924). The morphological entity is essentially cosmopolitan in distribution between 60N and 50S having been reported from North and South Africa, North and South America, Europe, Asia, India, and Australia but is absent from Japan (Braun and Nordstedt 1882; Corillion 1955, 1957a, 1957b; Groves and Bullock-Webster 1924; Migula 1890; Olsen 1944; Sarma and Kahn 1965; Wood 1965; Zaneveld 1940).

C. contraria is closely related to Chara vulgaris L.. Wood's recent revision of the Characeae (1965) treats C. contraria as a conspecific form of C. vulgaris. Primarily as a matter of personal preference, C. contraria is here treated as a distinct species following Zaneveld (1940), Groves and Bullock-Webster (1924) and Corillion (1957).

The diagnostic characters most frequently used to separate the two are 1) relative size of primary and secondary corticating cells on the main axis; 2) ratio of bracteole length to oogonia length; 3) length of stipulodes to width of main axis; 4) color of mature oospores. C. contraria exhibits the tylocanthous condition, i.e. the primary corticating cells are larger than the secondaries whereas the aulocanthous condition prevails in C. vulgaris with the primaries and secondaries being of equal size. The bracteoles and anterior bract cells of C. vulgaris usually are 6x to 10x the length of the mature oogonium while the bracteoles and anterior bracts of C. contraria vary from 1/2x to 3x the oogonium length in actively growing cultures. The stipulodes tend to range from 1/2x to 3/4x the diameter of the main axis in C. contraria while the stipulodes in C. vulgaris are longer and range from 3/4x to 1 1/2x the main axis diameter. Brown oospores frequently occur in

C. vulgaris while C. contraria oospores are virtually always black. Wood and Meunscher (1956) have emphasized the difficulty encountered in attempting to separate the two morphologically.

Since Kützing accorded C. contraria species status in 1845, the taxon has alternately been lowered to varietal or form level followed by reinstatement of full species status (Boswell 1884, Cosson and Germain de Saint-Pierre 1861, Migula 1890, Wood and Meunscher 1956). The long list of synonomies provided by Wood (1965) is eloquent evidence of the unstable classification efforts which have been based almost exclusively upon morphological approaches.

MATERIALS AND METHODS

To document the occurrence and delineate the local distribution patterns, in time and in space, of the various chromosome races found to occur in C. contraria, an intensive field effort was initiated to locate a large number of sites where the organism occurred. The two areas most thoroughly searched are indicated in Figs. 1 and 2. The Tatum, N.M. area consists of semi-arid range land with the only permanent water supply being livestock tanks fed by windmills. The Alpine, Texas area consists partly of the Davis mountains and surrounding foothills with the remainder being a dry plains area. Most permanent water is

again provided by windmills and stock tanks. The two regions are separated by a 450 km continuum of arid and semi-arid range land. Each tank which contained C. contraria was systematically sampled spatially and many on various dates during a period of 18 months. Single sprigs of C. contraria were isolated in the field, preferably when fertile and vigorously growing. The youngest tips of these sprigs, with developing antheridia, were removed and placed in freshly prepared Carnoy's fixative (3:1 ethanol:glacial acetic acid). The remaining vegetative portion was retained in a water filled plastic bag and keyed to correspond to the fixed tips. Such vegetative sprigs remain alive for several months in a cool light room and afford a ready means of establishing clonal greenhouse cultures should the subsequent cytological examination of the removed tip prove interesting.

The fixed tips were removed from the fixative after ca. 24 hours and placed in 70% aqueous ethanol. Material so preserved and stored in a refrigerator at 3C remained useful for cytological purposes for several months. The chromosome counts were made on mitotic figures present in the young antheridial filaments. The nucleic acid was stained with propionic orcein dye and the resulting material then prepared for counting by standard squash technique (Sharma and Sharma 1965).

Using the vegetative sprigs mentioned above, clones of known chromosome number and point of origin were established in polyethylene lined aquaria containing sandy loam soil and tap water. Some cultures were grown in the greenhouse while others were placed in the open when the season permitted. Snails, ostracods, and cladocera were used as grazers to control growth of filamentous and unicellular forms of contaminating algae. Only young, actively growing cultures are suitable for crossing purposes. When signs of senescence appeared, sub-clones were routinely established. The chromosome numbers of these clones were recounted regularly and after each sub-cloning step.

The experimental breeding program utilized a selected group of clones established as above. The techniques used were those developed by McCracken, Proctor and Hotchkiss (1966). Briefly, this consists of 1) emasculating monoecious sprigs with finely honed dissecting needles, and 2) placing such functionally female sprigs in a common container with the desired sperm donor to permit crossing. Appropriate controls designed to detect premature fertilization (i.e. selfing) or parthenogenetic development were consistently employed.

Fertilization is indicated by darkening of the oospore wall, failure by bleaching and subsequent abortion of the oogonia (McCracken, Proctor and Hotchkiss 1966;

Proctor et al. 1967). Recently, Harrington (1969) has suggested that white oospores can give rise to germlings. I have collected large masses of plants from the field with white oogonia attached and attempted to isolate these to conduct germination studies. Such oogonia are extremely fragile and almost invariably disintegrate when touched even gently. Those few which have sufficient structural integrity to be isolated disintegrate upon drying and re-flooding. Many oospores appear to be fully white upon cursory examination but have the usual dark oospore wall within a heavy lime coat. Such incrusted oospores, of course, can germinate and give rise to germlings. I have obtained no evidence to indicate that oogonia with white walls ever give rise to progeny, but, to the contrary, have considerable evidence that they do not do so.

In those crosses where fertilization occurred, the darkened oospores were collected for subsequent germination studies. The functionally female sprigs with attached dark oospores were isolated and allowed to stand in water at about 28C for two weeks. The sprigs and oospores were then gently abraded with diatomaceous earth or fine sand and allowed to stand in 28C water for an additional two weeks. The oospores were then washed free of debris and stored in the wet (dark) in polyethylene packets at 2C for at least 60 days. Such a dormancy period has been shown to enhance germination (Proctor 1960). Following this

period, the oospores were counted and inoculated into replicate pint jars containing sandy loam soil and tap water. Each cross which resulted in oospore formation was represented by at least six replicate germination containers with a minimum total of forty-five oospores. Selfed oospores from each clone used in the breeding program were treated in this same manner with at least three replicates of ten oospores each. The germination containers were incubated in dim light at 20-30C. Each container was routinely inspected and the young germlings removed when they reached a height of about 1 cm. Such isolated plants were then recultured in individual glass aquaria. Care was exercised to prevent accidental removal of ungerminated oospores along with the young germlings. The plants derived from putative hybrid oospores were isolated in this manner, reared to maturity, and scored for growth rate, self-fertility, and chromosome number where possible. Representative germlings derived from selfed oospores were treated similarly.

RESULTS

Chromosome counts were made on 291 individual field samples collected from 43 locations. Figures 3-10 are photomicrographs and interpretations of typical karyotypes representing the three chromosome races encountered. Repeated counting of clonally established

material cultured in the greenhouse and subjected to varying day lengths, temperatures, and light intensities has shown these numbers to be stable. Repeated vegetative sub-cloning has not yielded any alteration in chromosome number. No evidence for the occurrence of aneuploidy in either natural or cultured populations was found.

The occurrence and distribution patterns of the chromosome races are displayed in Figs. 1 and 2. Both n=28 and n=42 cytotypes are well represented throughout both collection regions. Additional clones from Israel, California, Washington, Indiana, and Mexico were also available. Apparently, both 28's and 42's occur throughout North America and may well occur in most places where C. contraria is common. Israel is a notable exception; only n=28 plants matching the description of C. contraria have been found. At seven locations, n=28 and n=42 plants were found growing intermingled in the same windmill tank. Location 615 yielded a single n=14 plant which remains in culture. This plant is morphologically very close to "typical" C. contraria but also resembles C. vulgaris. Despite repeated, intensive sampling in the same tank, as well as many surrounding ones, no additional n=14 plants could be found. Clone 615-C is the only n=14 plant I have seen which can be assigned to the morphological entity, C. contraria.

Examination of the external morphology of large numbers of plants having different chromosome numbers and different points of origin has failed to yield any consistent distinguishing character or group of characters by which the different cytotypes can be distinguished. This does not exclude the possibility that refined techniques, e.g. numerical analysis, might be able to separate them on a statistical basis but presently it is not possible to categorize newly collected field specimens with respect to either geographic area of origin or chromosome number without actual cytological examination.

The clones selected for the breeding program are listed in Table I. Voucher specimens for each of these clones will be deposited in the herbaria of the New York and Berlin Botanical Gardens. Each clone number represents a single collection site and subsequent letters indicate different samples from that particular tank. Thus, 615-A and 615-C represent two different plants, in this case having different chromosome numbers, collected from tank number 615. The X prefix, where it occurs, is part of the location number.

The results of the crossing program are listed in Table II, column 2. The left hand clone number represents the plant rendered functionally female and the right corresponds to the sperm donor. The first five crosses listed involved plants having the same chromosome number

but collected at sites separated by varying distances. All crosses were uniformly successful to the point of oospore darkening--which occurred readily.

The next eight crosses involved the n=14 plant from Tatum. The plant successfully functioned as a sperm donor to both n=28 and n=42 sprigs of C. contraria, although such fertilization occurred slowly. The crosses in this section were repeated numerous times in order to obtain a quantity of oospores sufficient to conduct the subsequent germination studies. When the 615-C plant was rendered functionally female and placed with the C. contraria clones, fertilization did not occur. On the other hand, when the n=14 plant was crossed to X-131, an n=14 C. vulgaris form, fertilization occurred in both directions. Additional work will be necessary to determine the relationships between the n=14 Tatum plant, C. vulgaris and C. contraria.

The final twelve crosses listed in Table II were between plants having numbers of 28 and 42. Eight of these crosses were successful and resulted in mature oospores while four failed completely. Note that all four crosses which failed were between plants collected at least 3000 km apart and which had different chromosome numbers. However, the X-188 x X-159 cross was successful and the distance separating points of origin in this cross was 15,500 km; the reciprocal X-159 x X-188 cross failed.

TABLE I

BREEDING CLONES ESTABLISHED

CLONE NR.	COLLECTION AREA	CHROMOSOME NR.	SELFED OOSPORE GERMINATION %
604-A	Tatum, N.M.	n=28	20
609-A	Tatum, N.M.	n=42	17
611-A	Tatum, N.M.	n=28	20
611-E	Tatum, N.M.	n=42	15
613	Tatum, N.M.	n=28	13
613-C	Tatum, N.M.	n=42	12
615-A	Tatum, N.M.	n=42	12
615-C	Tatum, N.M.	n=14	13
628	Alpine, Tex.	n=28	26
637-C	Alpine, Tex.	n=42	20
X-131*	Michigan	n=14	-
X-145	Indiana	n=42	17
X-159	Israel	n=28	12
X-188	California	n=42	17
X-191	Mexico	n=28	12

*Denotes C. vulgaris; Voucher specimens for all clones listed will be deposited in the New York and Berlin Botanical Gardens.

TABLE II

CROSSING AND GERMINATION DATA

CROSS	FERTILIZATION	GERMLINGS	COMMENTS
Like Chromosome Nr.			
611-A x 604-A n=28 n=28	+	7	Normal, fertile All n=28
604-A x 611-A n=28 n=28	+	6	Normal, fertile All n=28
637-C x 609-A n=42 n=42	+	3	Normal, fertile All n=42
609-A x 637-C n=42 n=42	+	7	Partially sterile All n=42
X-188 x 637-C n=42 n=42	+	2	Normal, fertile All n=42
X-131* x 615-C n=14 n=14	+	0	
615-C x X-131* n=14 n=14	+	0	

TABLE II--Continued

CROSS	FERTILIZATION	GERMLINGS	COMMENTS
Unlike Chromosome Nr.			
628 x 615-C n=28 n=14	+	8	See text All n=28
637-C x 615-C n=42 n=14	+	0	
615-A x 615-C n=42 n=14	+	0	
615-C x 628 n=14 n=28	-		
615-C x 637-C n=14 n=42	-		
615-C x 615-A n=14 n=42	-		
609-A x 604-A n=42 n=28	+	2	Sterile dwarfs No counts possible
604-A x 609-A n=28 n=42	+	0	

TABLE II--Continued

CROSS	FERTILIZATION	GERMLINGS	COMMENTS
628 x 613-C n=28 n=42	+	0	
611-A x 611-E n=28 n=42	+	0	
613-C x 613 n=42 n=28	+	0	
613 x 613-C n=28 n=42	+	1	Sterile dwarf No count possible
X-188 x X-159 n=42 n=28	+	0	
X-159 x X-188 n=28 n=42	-		
X-159 x 611-E n=28 n=42	-		
X-191 x X-145 n=28 n=42	-		
X-159 x 609-A n=28 n=42	-		

*Denotes C. vulgaris; Voucher specimens for all germlings except the sterile dwarfs will be deposited in the New York and Berlin Botanical Gardens.

The results of the germination study, using oospores derived from the crossing program are presented in Table II, columns 3 and 4. The germination data for the selfed oospores of the breeding clones are presented in Table I, column 4. These numbers are to be considered as approximations as many factors--some known, some unknown--affect the precise germination percentages.

All crosses between plants having the same chromosome number yielded apparently normal fertile progeny, with one exception, and the offspring all had the same chromosome number as the parental types without exception. The 609-A x 637-C cross yielded germlings which were vegetatively slightly slow growing and showed varying degrees of partial self-sterility.

Of the five crosses involving the n=14 plant which were positive, only one has yielded germlings. All eight progeny which were obtained had a chromosome number of n=28, the same as the maternal parent. All were fully self-fertile and vigorous growers. Careful examination of the morphology of this series of plants showed them to be indistinguishable from each other and from the maternal parent but quite distinct from the putative paternal parent. It is my judgment that these plants were derived from oospores which had been prematurely self-fertilized and thus do not represent hybrid offspring. The possibility of some sort of pseudogamy

cannot be excluded although it is not probable.

Only two of the seven crosses between plants having 28 or 42 chromosomes gave germination. The oospores from the 609-A x 604-A cross yielded two plants both of which were misshapen dwarfs and the 613 x 613-C cross yielded one dwarfed offspring. These plants emerged, were isolated and given careful attention but survived for only about two months. They reached a height of about 1-2 cm, presumably ceasing growth when the reserve food supply of the oospore was exhausted. Normal plants routinely reached heights of one meter or more in comparable time periods. The dwarfs were composed of only a few poorly differentiated cells and no chromosome counts could be made.

DISCUSSION

This study has investigated the occurrence and significance of chromosome races in local populations of C. contraria with the aim of clarifying the nature of the evolutionary unit within the genus. The results obtained have shown that 1) at least two morphologically indistinguishable, but apparently polyploid cytotypes exist, 2) such chromosome races occur sympatrically and in random distribution, 3) complete reproductive isolation exists between the respective cytotypes, 4) at least partial reproductive isolation can occur between plants

having identical chromosome numbers even when the points of origin are separated by only 400 km and 5) that the morphological entities C. contraria and C. vulgaris may not be completely isolated from each other. C. contraria, then, is typical of the genus in general, in that it consists of a series of reproductive isolates, the boundaries of which often do not coincide with boundaries drawn by investigators using external morphological characters. An examination of factors which probably contributed to the formation of these characteristics and an evaluation of some of the consequences seems appropriate.

The frequent and widespread occurrence of the two apparently polyploid cytotypes in C. contraria and in other "species complexes" (Proctor 1970, Proctor in press) indicates that polyploidy is widespread within the genus and thus may be of considerable evolutionary significance. Although there are reports to the contrary (Tuttle 1924), most available evidence supports the view that the charophytes have a haplobiontic life cycle (Oehlkers 1916; Pickett-Heaps 1967a, 1967b; Shen 1966, 1967a, 1967b; Sundaralingham 1946, 1954). Since the oospore is the only diploid stage, the major selection pressures are likely operative on the gametophyte generation. Additionally, these plants are frequent, and in many situations exclusive, inbreeders. Since the

egg and sperm are of identical genotypes (except for somatic crossing over, point mutation, etc.) sources of genetic variation open to such sexual systems are severely limited as compared to diploid or dioecious forms. Polyploidy is one mechanism which can contribute to variation within and between such populations (Stebbins 1950). Klekowsky and Baker (1966) have found polyploid to be an important feature of fern biology, perhaps by allowing the accumulation of mutations and variability which could not otherwise be tolerated by the organism. Similar reasoning may be applicable to the charophytes.

The divergence of ranges between two or more polyploid forms is well documented in higher plants (Stebbins 1950). In contrast, the cytotypes of C. contraria occur in complete sympatry. Evidence for the divergence of geographic ranges for these two forms is lacking with one possible exception; only the n=28 chromosome race corresponding to the morphological entity C. contraria has been found in Israel.

The apparently random distribution of the two races suggests that chance may play an important role in charophyte distribution. Whether a given tank will contain 28's and 42's or both appears to depend upon which, if either, first succeeds in establishing itself. Once established, a given cytotype may remain dominant.

Mixed populations probably result when both races are introduced simultaneously into a new tank and neither can exclude the other. The failure of these two chromosome races to separate geographically suggests that the genetic requirements with respect to environmental optima are quite similar.

The results of the breeding and germination study involving plants having the same chromosome number also support this view. With one exception, crosses between plants having identical chromosome numbers gave rise to apparently normal, fertile progeny--each with the parental chromosome number. Such results demonstrate that charophytes are genetically capable of outcrossing and that gene flow could potentially occur, even between plants collected 1500 km apart. The consistent vigor of progeny derived from crosses of plants between both collection regions (having identical chromosome number) suggests that the Tatum and Alpine areas consist of populations which are compatible genetically. In contrast, the partially sterile plants derived from the 609-A x 637-C cross demonstrate that barriers to exchange of genetic information can occur across relatively short distances. Although some plants from various tanks in a given region may well be able to interbreed, such interchange probably occurs only rarely.

The ecology of the windmill tank environment supports this view. For an established population of Chara to receive genetic input from plants growing in a different tank, at least three events must transpire: 1) the propagule, usually the oospore, must be transported between the two tanks, 2) the oospore must germinate and 3) the germling must survive to maturity and produce gametes. Then, and only then, can exchange take place regardless of genetic compatability. The first prerequisite can be accomplished by ducks as shown by Proctor and others (Proctor, Malone, and DeVlaming 1967; DeVlaming and Proctor 1968; Proctor 1968). How frequently this actually occurs is not known. The second requirement is quite stringent. If the oospore arrives at a time when the extant population is actively growing, it will be inhibited from germinating. In greenhouse cultures, it has been observed repeatedly that mature oospores rarely, if ever, germinate under actively growing clones. Harrington (1969) has recently confirmed these observations and suggests that a chemical inhibitor is involved. If the oospore arrives when no growth is occurring, it then faces difficulties suggested by problem three. The tank which already contains an established Chara population has literally millions of mature oospores settled on the bottom capable of germination. Even if the transported oospore can germinate as well as the resident

population, the competition to become established must be quite severe. The several thousand germlings derived from resident oospores significantly reduce the chances for successful competition by incoming Chara. Only in those rare instances where the incoming oospore develops to full maturity can genetic exchange occur. Even then, colonization is likely to come from nearby tanks which may be genetically similar. Obviously, when a new tank is built, these particular barriers to colonization do not exist and the incoming propagules can establish themselves more readily. The rapidity with which Chara appears in newly constructed tanks is evidence that such colonization and dispersal occurs. The successful crossing capability demonstrated above suggests that populations confined to a given region tend to be compatible genetically.

The apparent absence of consistent, distinguishing morphological characters by which plants can be categorized with respect to geographic point of origin or chromosome number, superficially suggests that C. contraria is truly cosmopolitan and that all forms are conspecific. Indeed, this is the position taken by Wood and others (Zaneveld 1940, Wood 1965). However, the results of the present experimental breeding and germination study clearly show that complete reproductive isolation can and does exist even in the absence of

concomitant morphological discontinuities. Seven attempted crosses in this study did not go even to the extent of black oospore formation demonstrating the existence of complete reproductive barriers between the respective clones. Note that all of these attempts involved plants having different chromosome numbers.

Clearly, negative results in the crossing attempts mean complete reproductive isolation. On the other hand, formation of black oospores does not constitute proof of potential gene flow between respective clones. It must be shown that such oospores will germinate and that fertile, vigorous progeny can be derived from them. The disparity between the results obtained at the crossing stage versus the germination stage are well illustrated in the 28 and 42 series of crosses. Twelve crossing attempts were successful to the point of fertilization but none of the oospores so derived have given rise to fertile hybrids. Two crosses yielded prostrate, misshapen dwarfs which failed to achieve significant differentiation. Such plants could not likely survive under natural conditions and certainly could not contribute genetically to subsequent generations. The remaining ten crosses resulted in oospores which did not germinate. It is important to note that either a zero germination percentage or the production of sterile dwarfs only is just as effective in isolating

two populations as is failure to effect fertilization. Having a different chromosome number is a sufficient but not a necessary condition for the existence of a complete reproductive barrier. Increasing the geographic distance between collection sites reduces the chance of gene flow and increases the chance of genetic incompatability even between plants having the same chromosome number--although this is not necessarily the case (Proctor in press).

The occurrence of reproductive isolates and different chromosome races in unpredictable patterns suggests that chance may also play an important role in genetic divergence among the charophytes. The role of chance in evolution has been discussed in various contexts (Wright 1931, Talling 1951, Mayr 1963). As noted above, the apparently random but sympatric distribution of 28's and 42's in C. contraria indicates that either or both can become established in a given tank depending primarily upon the vagaries of dispersal. Once stabilized, the Chara population in a given tank may become essentially closed genetically with little, if any, gene immigration occurring. When this is true, each such tank then becomes an isolated "evolutionary unit." Such spatial isolates would be expected to diverge from other such units through chromosomal rearrangements and other mutational mechanisms as a function of

time. Again, chance probably becomes an important feature of such divergence. Since these windmill tanks are composed of small numbers of genotypes--frequently only one--and because of the charophyte's tendency to inbreed, genetic drift or the Sewall Wright effect (Mayr 1963, Wright 1931) could be an important contributing factor.

The existence of several graded degrees of reproductive incompatability (Proctor in press, Proctor submitted) reflecting different stages of divergence supports the view that small, local populations are the "units of evolution" in the charophytes and indicates that such populations are currently in an active stage of genetic divergence.

Obviously, the taxonomy of such a complex and dynamic situation is difficult and necessarily less than clear cut. In his recent revision of the Characeae, Wood (1965) recognized the existence of many phenotypes and genotypes below the species level, but, unfortunately I think, chose to submerge and minimize such intricacies by defining the species in a very broad morphological manner. It is precisely those aspects of charophyte biology, namely: 1) the existence of a large number of reproductive isolates, 2) the absence of consistent, morphological discontinuities which coincide with reproductive barriers, 3) the existence of intergrades showing

varying degrees of genetic incompatability, and 4) the dynamic and active nature of the isolating processes which, at the same time, render taxonomic treatment difficult and are potentially the most useful as tools and models for evolutionary research. Thus any classification scheme should recognize and accept--if not emphasize--the complex nature of the charophyte species.

SUMMARY

In C. contraria, two apparently polyploid cytotypes with haploid numbers of n=28 and n=42 have a widespread and frequent occurrence. The two occur sympatrically and are morphologically indistinguishable. Their unpredictable pattern of distribution suggests that chance plays an important role in charophyte dispersal and colonization.

Experimental breeding and germination studies have demonstrated that plants with identical chromosome numbers can cross and give rise to apparently normal, fertile progeny. However, partial reproductive barriers between such plants have been demonstrated even when originating from points only 400 km apart.

Essentially complete reproductive barriers exist between plants having different chromosome numbers. Crosses between such plants sometimes produced dark oospores, usually dependent upon the distance between

collection sites. Such oospores, when formed, either did not germinate or produced sterile dwarfs which could not contribute genetically to subsequent generations. Such data clearly show that black oospore formation is not proof of potential gene flow.

C. contraria appears to be typical of several "species complexes" within the genus in that it consists of a large number of morphologically unidentifiable reproductive isolates. Such isolates are thought to arise as a result of chromosomal rearrangements and other mutational mechanisms in small, local populations.

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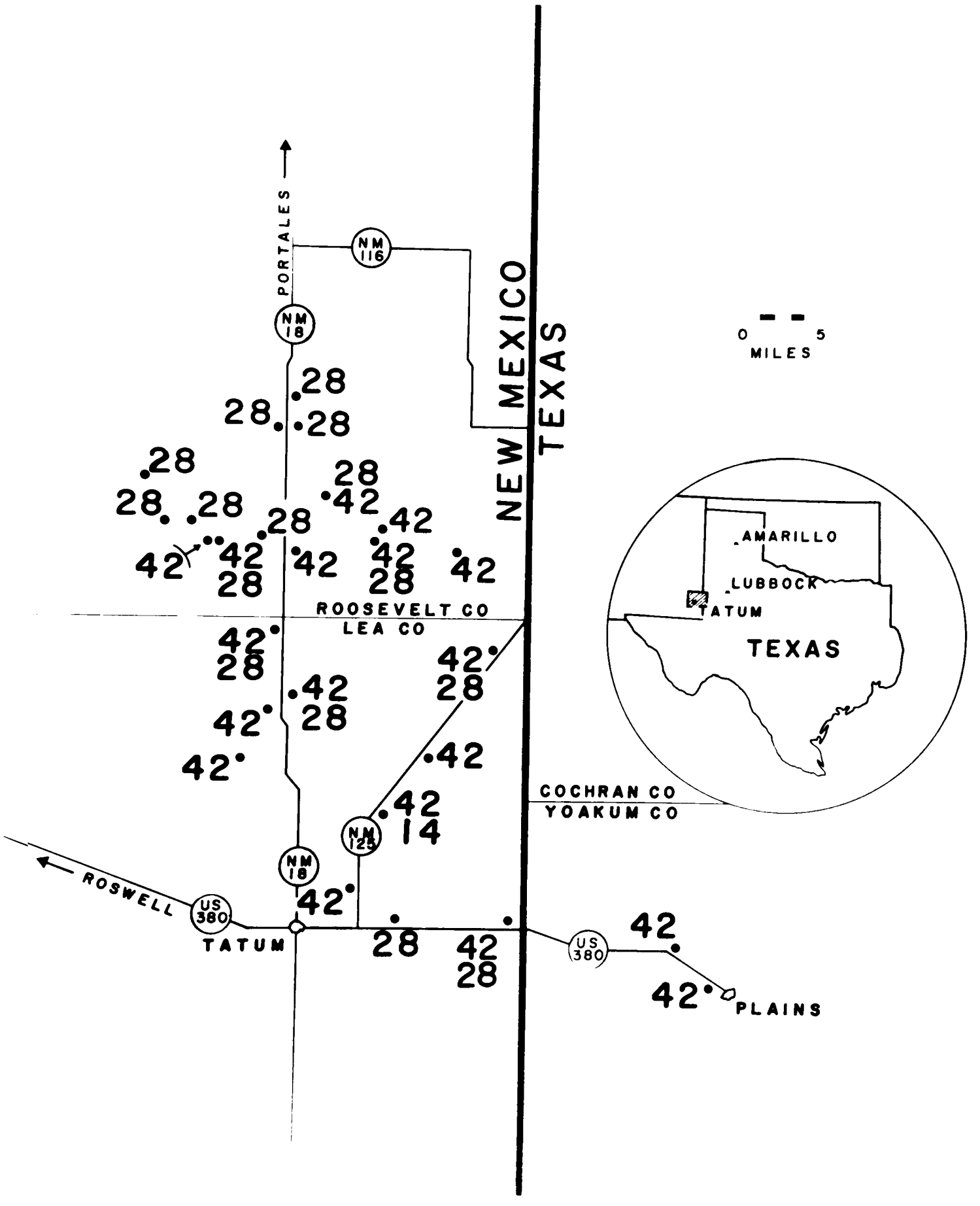
APPENDIX

Figure 1.--Distribution map of chromosome races in C. contraria, Tatum, New Mexico region.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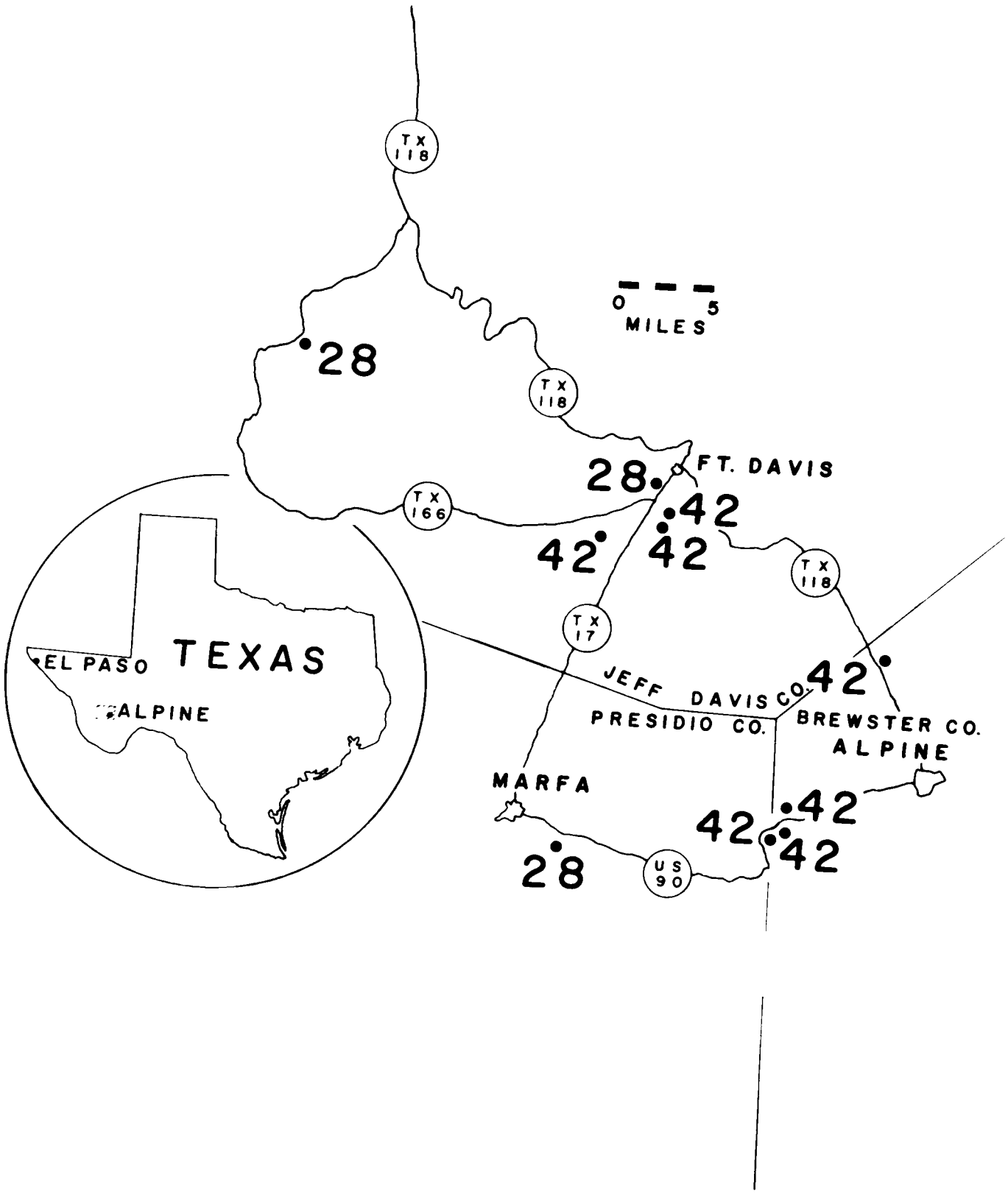
NM 18
NEW MEXICO
TEXAS
28
28
28
28
28
28
42
42
42
42
42
42
42
28
28
28
ROOSEVELT CO
LEA CO
42
28
42
28
42
28
42
42
42
28
42
28
42
14
NM 125
NM 18
ROSWELL
US 380
TATUM
28
42
28
US 380
42
42
PLAINS
COCHRAN CO
YOAKUM CO
0
5
MILES
AMARILLO
LUBBOCK
TATUM
TEXAS

Figure 2.--Distribution map of chromosome races in C. contraria, Alpine, Texas region.


TX 118
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28
TX 118
28
FT. DAVIS
TX 166
42
42
42
TX 118
TX 17
JEFF DAVIS CO.
42
PRESIDIO CO.
BREWSTER CO.
ALPINE
MARFA
42
42
42
28
US 90
EL PASO
TEXAS
ALPINE

Figure 3.--Photomicrograph I of clone 613-C karyotype (n=42). Bar is 15u long.

Figure 4.--Author's schematic interpretation of Figure 3.

Figure 5.--Photomicrograph II of clone 613-C karyotype (n=42). Bar is 15u long.

Figure 6.--Author's schematic interpretation of Figure 5.

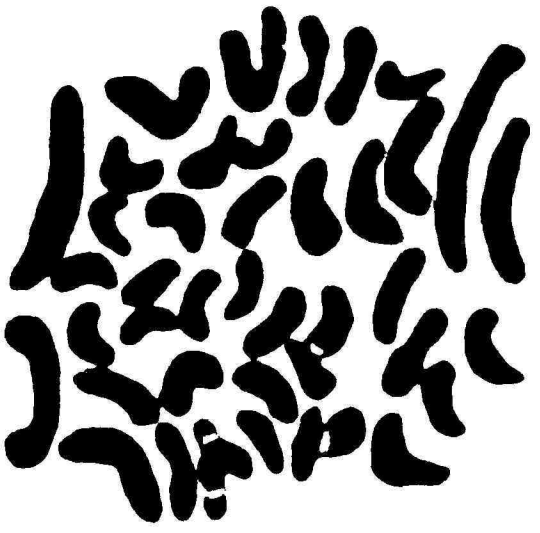

Figure 7.--Photomicrograph of clone 613 karyotype (n=28). Bar is 15u long.

Figure 8.--Author's schematic interpretation of Figure 7.

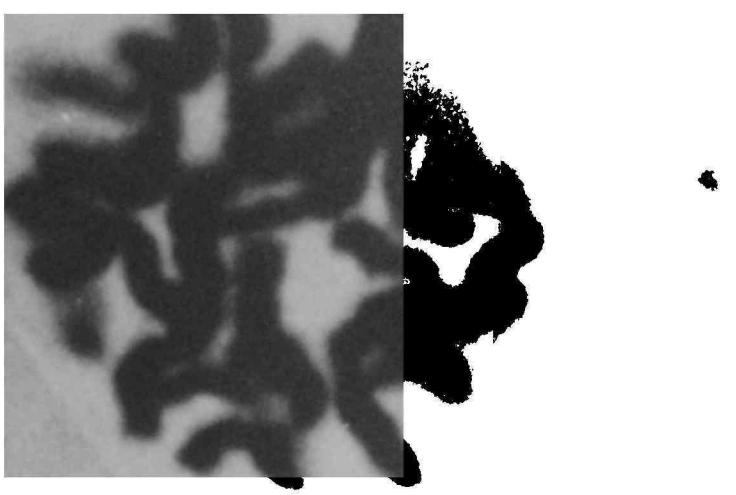

Figure 9.--Photomicrograph of clone 615-C karyotype (n=14). Bar is 15u long.

Figure 10.--Author's schematic interpretation of Figure 9.