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Karyotypes of Hydrilla (Hydrocharitaceae) Populations in the United States

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ABSTRACT

Standardized chromosome lengths and arm ratios of hydrilla collected from eleven geographically distinct populations in the United States were not significantly different. The karyotype of these populations which consisted of 5 acrocentric, 2 submetacentric and 1 metacentric chromosomes that ranged in total length from 1.69-5.54 μm was similar to previously published karyotypes. The triploid number of 24 chromosomes was observed in populations where only diploids had previously been reported and endopolyploidy is suggested.

Key words: Chromosomes, genetics, diploid, triploid, endopolyploidy, monoecious, dioecious.

INTRODUCTION

Polymorphism and genetic variation of hydrilla [*Hydrilla verticillata* (L.f.) Royle] have been discussed in reviews by Pieterse (1981) and Cook and Lüönd (1982). According to Cook and Lüönd (1982):

"Some races of *H. verticillata* seem to be dioecious, others monoecious. In climatically 'tropical' regions the plants are usually monoecious with male and female flowers at separate whorls, often on separate branches, in temperate regions the plants appear to be dioecious. However, in cultivation plants may develop one sex for one or two seasons and then revert to the other sex a season later. This observation sheds some doubt on the truly dioecious nature of some races, however, European material has been observed in the wild and in cultivation since ca. 1830 and it has never yet developed male flowers."

In the subtropical climate of Florida, where hydrilla has been studied for 30 years, staminate flowers have never been observed, and it was believed that only female hydrilla existed in the United States. However, staminate flower production by hydrilla that had been collected from Washington, D.C. (Vandiver et al. 1982), and Delaware (Steward, 1983, personal communication) was reported under experimental conditions in Florida. Likewise, in the summer of 1983, profuse staminate flower production was observed in monoecious hydrilla populations in North Carolina lakes (Langeland and Schiller, 1983).

Prolific vegetative reproduction, aided by man's transportation of vegetative material, has allowed rapid spread of hydrilla. However, this means of dispersal is most effective over short distances under natural conditions. The occurrence of monoecious hydrilla in the United States suggests the potential for viable seed production which is a natural mechanism for long distance dispersal. Conant et al. (1984), have observed seed production and germination under experimental conditions by hydrilla collected from Delaware and North Carolina. Hydrilla was observed to produce viable seed in North Carolina lakes (Langeland and Smith, 1984) but the viability of seeds and seedlings was low (Langeland and Smith, 1988). This low viability may be a result of defective sex cell formation because Harlan et al. (1984) have reported that hydrilla populations in North Carolina are triploid ($2n = 3x = 24$).

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Hydrilla populations have been compared genetically by chromosome number and by isoenzyme analysis. Hydrilla collected from Washington, D.C. had several isoenzyme characteristics that differed from hydrilla collected from other areas of the United States (two California collections, three Florida collections and one from Texas) (Verkliej et al. 1983a). Hydrilla collected from Washington, D.C., Maryland, and Delaware also was reported as having 16 (2n = 2x) chromosomes compared to 24 (2n = 3x) for all other U.S. collections (Verkleij et al. 1983b). It was assumed that hydrilla populations in North Carolina, which are similar in morphology and sexual expression (monoecious) to populations centered around Washington D. C., were of the same strain. However, Harlan et al. (1984) reported that hydrilla collected from North Carolina (Lake Wheeler) was triploid (2n = 3x = 24). Chromosome numbers of 42 hydrilla plants had been published as of 1981. Of these, 21 were diploid (2n = 2x = 16), 20 were triploid (2n = 3x = 24) and 1 was tetraploid (2n = 4x = 32) (Cook and Luond, 1982). Diploid and triploid populations have been on record since 1929 (Sinoto, 1929), whereas the tetraploid was recently reported (Davenport, 1980).

Published idiograms of diploid hydrilla have 3 short, 2 medium and 3 long chromosomes. Triploid karyotypes are less consistent (Cook and Luond, 1982). One published triploid karyotype records 5 long and 3 short chromosomes (Sinoto, 1929), while another reports the triploid idiogram corresponds with the diploid aside from the extra set of chromosomes (Czapik, 1978). Metaphase chromosomes range in length from 2.3 to 2.5 μm for the smallest to about 7.5 to 8.0 μm for the longest (Pieterse, 1981; Sharma and Bhattacharyya, 1956; Czapik, 1978). Sinoto (1929) suggested that an unequal pair of chromosomes (XY sex chromosomes) was responsible for sexual expression. However, there has been no subsequent evidence for the presence of sex chromosomes (Cook and Lüönd, 1982). Likewise, there has been no verification of a relationship between vegetative morphology or ecology and karyotype (Verkleij *et al.* 1983a; Chaudhuri and Sharma, 1978; Czapik, 1978) as was suggested by Misra (1966, 1971).

The purpose of this study was to determine if hydrilla populations in the United States can be distinguished by karyotyping. This information may be helpful toward explaining seed and seedling viability of monoecious hydrilla populations.

MATERIALS AND METHODS

Hydrilla collected from eleven geographically separate populations in the United States (Table 1) was cultured in a greenhouse and root tips were collected from these cultures for karyotyping. Root tips were harvested between 7 and 9 am for obtaining maximum metaphase images. Root tips were pretreated in 8-hydroxyquinoline for 2-4 hours. After pretreatment, the root tips were rinsed in distilled water and placed in 3:1 ethanol/acetic acid at room temperature for a minimum of 4-24 hours. Root tips were then macerated in 1N HCl at 60 C for approximately 10 minutes and rinsed. DNA was stained by soaking root tips overnight in Leuco-basic fuchsin and then rinsing in distilled water. A root tip section 1 mm in length was placed on a microscope slide with a drop of 45% acetic acid. A cover slip was placed on the slide and the eraser end of a pencil was used for initial spread of the cells. The slide was then pressed at 5000 psi on a Carver lab press. A dry ice method was used to make permanent slides.

TABLE 1. COLLECTING LOCATIONS, COLLECTORS, AND NUMBERS OF CELLS USED FOR KARYOTYPING HYDRILLA POPULATIONS IN THE UNITED STATES.

Location	Collector-Date	Total No. of cells Karyotyped
Constitution Gardens Washington D.C.	R. R. Yeo—[1]	10
Dyke Marsh, VA	C. F. Reed—11/85	2
Fort Lauderdale, FL (culture)	D. L. Sutton—9/84	2
Guntersville, Resovoir Jackson Co. AL	E. R. Burns—10/84	1
Kenilworth Aquatic Gardens, Washington, D.C.	K. A. Langeland—10/85	3
Lake Kerr, FL	K. A. Langeland—12/84	2
Lake Raleigh, NC	C. B. Smith—10/84	2
Lake Wheeler, NC	C. B. Smith—10/84	5
Lilypons Aquatic Gardens, MD	K. A. Langeland—10/85	1
Lilypons Aquatic Gardens, TX	W. T. Haller—[2]	2
Violets Lock, MD	R. R. Yeo—[1]	1

[1]Received 5/85, collection date uncertain.
[2]Received 12/84, collection date uncertain.

Cells that were photographed and used for karyotyping were catalogued by recording the slide and negative frame number on the film. Chromosome images were cut from the prints and visually arranged into eight groups of homologues (x = 8). Arm lengths were measured on prints and corrected for magnification. Total chromosome lengths, arm ratios (short arm length/long arm length), centromere indices (short arm length/total chromosome length), and standardized lengths (chromosome length/longest chromosome length in the cell) were calculated. Chromosome groups were then plotted by standardized length and arm ratio to detect and correct inaccuracies in visual groupings. Homologous groups were compared among populations with respect to standardized length and arm ratio using multivariate analysis (SAS 1985).

RESULTS AND DISCUSSION

The triploid chromosome number (2n = 3x = 24) was observed in all populations in this study. This agrees with Harlan *et al.* (1984) who reported that monoecious hydrilla collected from Lake Wheeler, NC was triploid (2n = 3x = 24). However, our observations conflict with those of Verkleij *et al.* (1983b) who described diploid (2n = 16) hydrilla collected from "Washington D. C., Re-

flecting pool; Washington D. C., Kenilworth Gardens; and Maryland, Lilypons Gardens". These were most likely representatives of the same populations used in our study. This discrepancy may suggest the existence of mixed populations of diploid and triploid individuals and/or endopolyploidy in hydrilla root tips. In the former case, random collections could result in obtaining either diploid or triploid karyotypes. Although the probability is low, it is possible for triploid parent plants in the populations to produce diploid progeny, in addition to progeny with other ploidies. Diploid progeny would be viable, whereas embryos with one to several extra chromosomes would probably not be viable, or result in low seedling vigor, as observed by Langeland and Smith (1988). Mixed populations could also result if triploid progeny are produced by diploid parents from unreduced gametes. In the latter case, either the diploid or triploid chromosome number (or other ploidy) could be observed in the same collection or individual. Chimeras have previously been suggested in the Hydrocharitaceae (Sharma and Bhattacharyya, 1956). Although insufficient cells were observed in this study to determine endopolyploidy, further investigations are being conducted to verify this.

All cells used in this study for karyotyping were triploid. Therefore, chromosomes were compared among populations as eight groups of three homologues (Figure 1 and Table 2). Chromosomes within groups were not significantly different among populations with respect to standardized length and arm ratio according to Wilks' lambda, Pillai's trace, Hotelling-Lawley trace, or Roy's maximum root (SAS, 1985). Therefore, we concur with Chaudhuri and Sharma (1978) that karyotypes are not correlated with morphology or ecology. Differences in length with respect to ecology, as suggested by Misra (1966, 1971) would be difficult to demonstrate because of the variability in chromosome length within the same population and root tip. For example, in this study the length of the longest chromosome in a cell ranged from 4.25 μm to 8.38 μm in the same root tip. These differences are a result of the degree of condensation of chromosomes that was affected by the stage of metaphase and the fixation process.

Since chromosomes were not significantly different among populations, morphological characteristics of chromosomes were combined and grouped into a karyotype that was common to all populations (Figure 1 and Table 2). Chromosome groups 1 and 5 are distinctly acrocentric (arm ratio < 0.33) and separate distinctly from other groups by size (standardized length) and arm ratio (Figure 1 and 2). Groups 2-4 appear as a single natural grouping but they were separated into three separate groups to attain eight groups of three homologues among the 24 chromosomes. Group 2 tends to be longer than Groups 3 or 4 and less acrocentric than Group 3. Group 3 chromosomes tend to be longer and more acrocentric than Group 4. The nine chromosomes of groups 6-8 are similar in size but, despite some overlap, these groups appear to be distinct considering differences in arm ratio. Groups 6 and 8 are submetacentric (0.33 < arm ratio < 1.00), with group 6 having a smaller arm ratio. Group 7 is

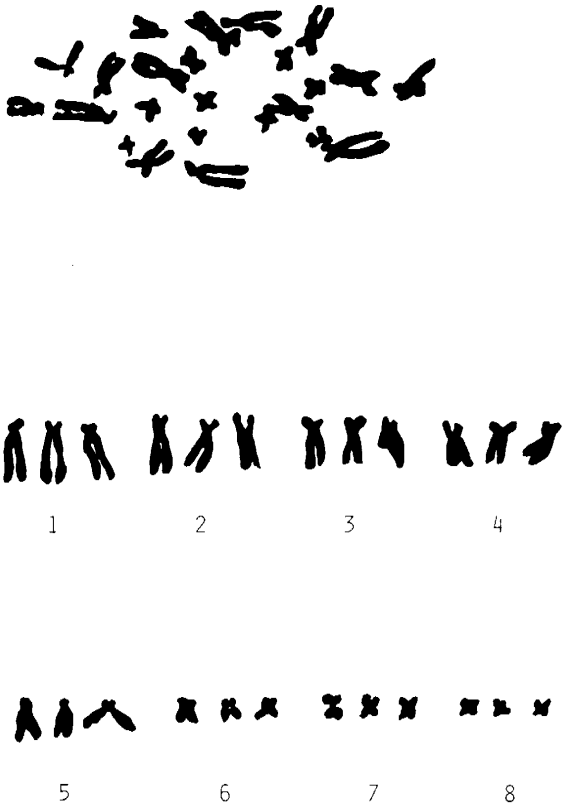

Figure 1. Early metaphase, somatic chromosomes of hydrilla collected from Lake Wheeler, North Carolina, 10/84.

TABLE 2. CHROMOSOME MORPHOLOGY OF HYDRILLA COPLLECTED FROM VARIOUS POPULATIONS IN THE UNITED STATES (n = 93).

Chromosome Group No.	Total Lengfth (μ)		Standardized[1] Length		Arm Ratio[2]	
	Average	Range	Average	Range	Average	Range
1	5.54(0.11)[3]	3.32-8.37	0.94(0.01)	0.77-1.00	0.22(0.00)	0.16-0.31
2	5.47(0.10)	3.32-7.50	0.93(0.01)	0.80-1.00	0.53(0.01)	0.43-0.65
3	4.97(0.10)	2.93-7.00	0.84(0.01)	0.69-0.95	0.41(0.01)	0.29-0.58
4	4.39(0.08)	2.54-6.12	0.75(0.01)	0.59-0.85	0.51(0.01)	0.32-0.70
5	3.51(0.06)	2.00-5.00	0.60(0.00)	0.44-0.74	0.26(0.00)	0.17-0.38
6	2.18(0.04)	1.30-3.25	0.37(0.00)	0.29-0.53	0.61(0.01)	0.42-1.00
7	2.12(0.04)	1.20-3.00	0.36(0.01)	0.26-0.53	0.98(0.00)	0.80-1.00
8	1.69().03)	0.90-2.25	0.29(0.00)	0.20-0.41	0.80(0.01)	0.50-1.00

[1]Chromosome length/length of longest chromosome in cell.
[2]Short arm length/long arm length.
[3]Numbers in parentheses are standard error of means.

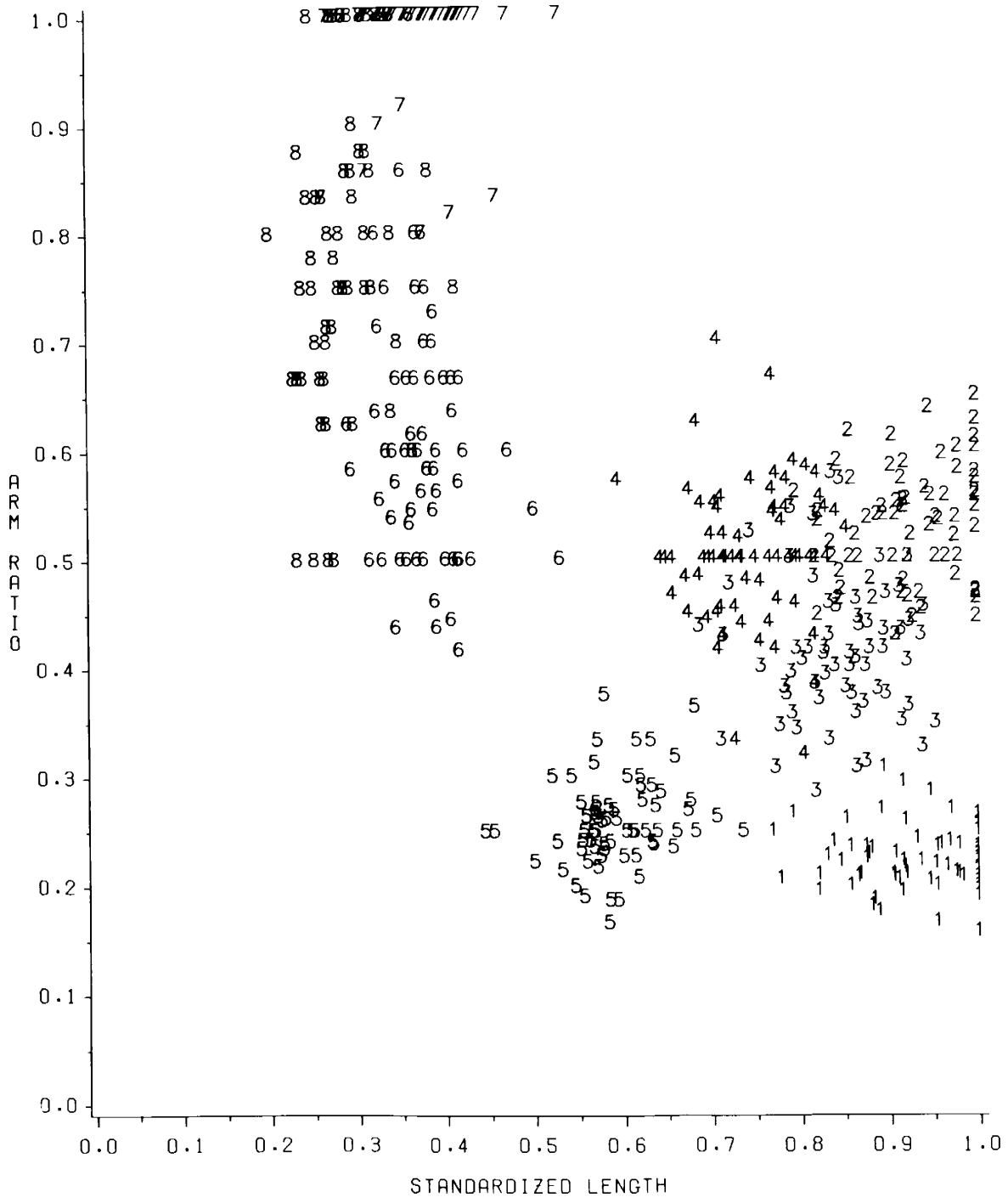

Figure 2. Scatter plot of arm ratios (short arm length/long arm length) and standardized lengths (chromosome length/length of largest chromosome in cell) of somatic chromosomes from eleven hydrilla populations in the United States.

metacentric (arm ratio = 1). Secondary constrictions, described by Sharma and Bhattacharyya (1956) or a pair of unequal chromosomes suggested by Sinoto (1929), were not identified in this study or by other researchers.

Chromosome morphology observed in this study was similar to that reported by others. Groups 1-5 and 6-8 correspond, respectively, to the 15 long and 9 short chromosomes reported by Sinoto (1929) for hydrilla from Itikawa, Japan; and groups 1-3, 4-5, and 6-8 correspond, respectively, to the 9 long, 6 medium, and 9 short chromosomes of female triploid plants from Japan. Our idiogram is similar in appearance to that published by Harada (1955), however, chromosome morphology was not quantified in that idiogram. Czapik (1978) quantified the idiogram of diploid hydrilla from Irish lakes and a triploid from Poland. Neither of these populations were observed to flower, but it was assumed that they were female because their idiograms were similar to those of female plants reported by Sinoto (1929). The chromosomes of the triploid, with 12 acrocentric, 9 submetacentric, and 3 metacentric chromo-

somes, corresponded closely with the diploid. The average size of chromosomes in the United States populations (1.67 μm to 5.54 μm; Table 2) was somewhat smaller than that of the diploids from Irish lakes (2.46 μm to 7.79 μ m). Chromosomes of the triploid from Poland were reportedly more condensed than the diploids which may explain the apparent smaller size chromosomes from the United States populations. The size of chromosomes in the Irish lake populations compare well with those of the United States populations in that the group sizes of both idiograms compare proportionately, and the sizes of those from the Irish lakes fall within the ranges observed in the United States populations. Although our idiogram differs from the Polish triploid (6 acrocentric, 15 submetacentric and 3 metacentric chromosomes) we feel that inaccuracies in visualizing and measuring chromosomes can explain these slight differences, and that the idiograms for hydrilla from Poland and the United States can be considered the same.

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Laboratory Host Range Studies With a Leaf-mining Duckweed Shore Fly

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ABSTRACT

A leaf-mining shore fly, *Lemnaphila scotlandae* Cresson (Diptera: Ephydridae), collected from duckweeds (*Lemna* spp.) in Florida was exposed in paired-choice oviposition tests to 19 aquatic macrophytes and 1 alga. Eggs were laid only on 6 species in the duckweed family, Lemnaceae. Larvae developed to adults only on 3 of those species, all duckweeds. In no-choice oviposition and fecundity tests, more eggs were laid on common duckweed (*Lemna minor* L.) than on inflated duckweed (*L. gibba* L.) and small duckweed (*L. valdiviana* Phil.). This shore fly can be considered a potential candidate for biocontrol of duckweeds in countries where it is not present.

Key words: Aquatic weeds, biological control, Diptera, Ephydridae, *Lemna*, oviposition tests.

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