NUMERICAL ANALYSIS OF PHENETIC VARIATION OF NATURAL POPULATIONS OF *TRADESCANTIA BRACTEATA*, *T. OHIENSIS* AND PUTATIVE HYBRIDS[1].

WU-TSANG CHENG[2]

Abstract: Discriminant analysis of five natural populations of *Tradescantia bracteata* (2n=12) and *T. ohiensis* (2n=24) showed that the two species are distinct in their phenetic characteristics and differ in their ecological preference. A tetraploid hybrid derivative population (2n=24) was found. It is phenetically closer to *T. ohiensis* than to *T. bracteata*. Chromosomal variations of populations were also examined.

INTRODUCTION

American tradescantias have been extensively studied taxonomically and cytologically (Anderson, 1954; Anderson and Sax, 1936; Anderson and Woodson, 1935). Morphologically they have been divided into four groups: *T. virginiana* and relatives, *T. micrantha*, *T. fluminensis* complex, and *T. commelioides*. Both diploids ($2n=12$) and tetraploids ($2n=24$) are found and eight species have both diploid and tetraploid races (Anderson and Sax, 1936). The *virginiana* group is characterized by self-sterility, inter-fertility and perennial habit and a great deal of natural hybridization among species. The natural hybridization results in great variation within species and has been the subject of many previous studies particularly by E. Anderson.

Anderson (1936a) proposed a hybrid index method which uses uncorrelated characters to measure the effect of hybridization of tradescantias at the population level and this method has been applied by many other authors. The diploid and tetraploid strains within a species are apparently indistinguishable morphologically. By studying artificial hybridization, Anderson (1936a, 1936b) concluded that the morphological consequences of inter-species hybrids of *Tradescantia* were very much affected by numerical relationship of chromosome number between the parents. The crossing between two tetraploids or two diploids produces intermediate hybrids, but crossing between tetraploid and diploid produces hybrids which are more like the tetraploid parents.

Natural hybrids of tradescantias are occasionally found in disturbed habitats (Anderson and Hubricht, 1938; Anderson and Woodson, 1936; Riley, 1937, 1939). Anderson hyphothesized that such disturbances cause a hybridization of habitats yielding an array of ecological niches which correspond to the preferences of inter-species hybrids. Analysis of the composition of these hybrid populations showed that hybrids tended to cross back to one of their parents. By this process the genes of one species incoporate into the gene pool of another. Anderson and Hubricht (1938) provided the name "introgressive hybridization" to this phenomennum. The present study is an analysis of phenetic and chromosomal variation of populations of *Tradescantia bracteata* Raf. and *T. ohiensis* Small, to evaluate the effect of hybridization.

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(2) 鄭武燦, Dept. of Botany, NTU, Taipei, R.O.C.

MATERIALS AND METHODS

1. Populations sampled.

Five populations of plants were collected for this study. Their code numbers and localities are listed in Table 1. The code number of each population will be applied throughout in this paper.

Population 1 is distributed sparsely among grasses and herbs in a prairie beside a country road. Population 2 is located along the outer bank of a roadside ditch extending about 150 m long and 1-1.5 m wide. Population 3 is a large population consisting of thousands of individuals, occupying a belt on a slope along a railroad track about 250 m long and 2-3 m wide. Almost all the tradescantias are in large clumps containing several scores of stems. Populations 4 and 5 are small and located on the north eastern and southern sides, respectively, of a hill. Population 4 occupied a spot at the hill side where in the afternoon it is under the shade of the forest.

Table 1. Localities of *Tradescantia* of populations and code number.

Population	Localities
1	Section 33, R19E, T12S. 1.5 miles west along highway 40 from Lawrence then south for 0.5 mile.
2	Section 24, R19E, T12S. 2 miles south along Louisiana Street.
3	Section 1, R19E, T12S. 3 miles north along highway 59, at junction with railroad.
4	Section 7, R19E, T12S. 4 miles west along highway 70 from Lawrence then north for 2 miles.
5	Section 13, R19E, T12S. 4 miles west along highway 70 from Lawrence then north for 1 mile.

Thirty individuals were sampled from populations 1, 2 and 3. Ten individuals were sampled from population 4 and four individuals from population 5.

Flowering time of each population was observed in the field. The recored was made weekly from early April to August. The average for 1973 and 1975 is showned on Table 2.

Table 2. The flowering period of natural populations, average for 1973 and 1975.

Population	early April	May	June	July late
1.		.....		
2.		.....		
3.	.....			
4.		.....		
5.	.....			

2. Method of scoring tne characters.

Twenty-two morphological characters were used and each was assigned a code number (Table 3) which will be applied in the following section in this paper. Degree of pubescence was obtained from pedicels and sepals, counting the number of hairs along the edges. The value used for each plant was the mean of three pedicels and sepals.

Table 3. Twenty-two characters measured, with code number, for population variation study in *Tradescantia bracteata*, *T. ohiensis* and putative hybrids.

Code number	Characters	Code number	Characters
1.	Plant height	12.	Width of the lower bract
2.	No. of flowering branches	13.	Length of the upper bract
3.	No. of vegetative branches	14.	Width of the upper bract
4.	No. of internodes of stem	15.	Mean length of pedicels
5.	Total No. of internodes	16.	Mean length of sepals
6.	Mean length of the three upper internodes	17.	Mean width of sepals
7.	Mean length of the three upper leaf blades	18.	Eglandular hairs on pedicel
8.	Mean width of the three upper leaf blades	19.	Glandular hairs on pedicel
9.	Mean length of the three upper sheathes	20.	Eglandular hairs on the apex of sepal
10.	Mean width of the three upper sheathes	21.	Glandular hairs on sepal
11.	Length of the lower bract	22.	Eglandular hairs on sepal

Fresh specimens were used for populations 1 to 4, whereas, pressed specimens were used for population 5. Metric characters of the pressed specimens from population 5 were multiplied by a correction factor of 1.1 obtained by comparing measurements from fresh materials with those of pressed specimens of population 1 to 4. The region above the uppermost node of the main stem, but not of the branches, was counted as one internode. Usually the means of three leaf blades, sheathes and internodes were measured. If there were only two nodes on an individual, two leaf blades, sheathes and internodes were measured.

In some cases, the uppermost node was extremely short apparently causing the upper two umbels to be mixed together and having three or four bracts subtending the inflorescences; in these cases the lower two bracts were measured.

3. Discriminant analysis.

The phenetic variation of natural populations can be examined in two ways, within population and between populations. There are several techniques to analyze these sources of variation, but discriminant function analysis was employed in the study. Discriminant analysis consists of finding a transformation which maximizes the intergroup differentiation with respect to intragroup differentiation. It is a technique to compress a large number of variables into fewer dimensions which explain most of the variation and can be projected into two or three dimensional models. To find the discriminant functions, we have to solve the following equation,

$$|B - zW| = 0,$$

where, B is the matrix of pooled variance-covariance between populations, W is the matrix of pooled variance-covariance within populations, and z, the coefficient of discriminant function equation, is a column vector.

When a set of z coefficients are obtained, they are entered in the discriminant function equation of the following form,

$$R = z_1x_1 + z_2x_2 + \cdots\cdots + z_mx_m,$$

where x is the sample mean, m is the number of characters and R is the discriminant score.

Individual specimens can be plotted by substituting the individual character's value in place of the sample mean.

There is a characteristic root for each discriminant function. Dividing a particular root by the sum of all roots gives an estimate of the percent of the variation explained by that discriminant function. Chi-square tests of significance can be used on each function.

Since a character which is invariant in a population cannot be applied to the discriminant function analysis, four of the twenty-two characters measured were not treated in this study. The first three functions which explained the greatest proportion of the variation were projected into a three dimension model. The generalized distance (Mahalonobis, 1936) was also applied in this study as an additional estimate of phenetic similarity. This is a weighted coefficient considering both correlation among characters and variance of seperate characters. It is defined by the following equation,

$$D^2 = b_1 d_1 + b_2 d_2 + \cdots\cdots + b_m d_m,$$

where b is the weights of the characters in the discriminant function, d is the difference between mean of each character in the two populations, and m is the number of characters. In this study the square root of D^2 was used. When the population means were projected onto the first three discriminant functions, they were connected by a straight line representing the lowest value in the generalized distance matrix.

All the above computations were carried out on a Honeywell 636 computer at the computer center of the University of Kansas. The programs used is MULDIS programs written by F. James Rohlf, John Kishpaugh and R.L. Bartcher.

4. Chromosome counts

Chromosome numbers of tradescantia plants in eastern Kansas were examined for the analysis of chromosomal variation of natural populations. Root-tips were collected in the field pretreated in 0.05% colchicine for 4 hours, fixed in absolute ethanol/glacial acetic acid (3:1) for 24 hours and stored in 70% ethanol at 4°C until they were used. The root-tips were squashed and stained in acetic-orcein after hydrolysis in 10% HCl for 15-20 minutes.

RESULTS

The mean values of the characters are shown in Table 4. Four characters were found to be discontinuous between populations. Character 5, the total number of internodes of branches, is absent from populations 1, 2 and 5. Character 18, eglandular hairs on pedicel, is absent from populations 1 and 2. Characters 19 and 21, the glandular hairs on pedicel and sepal, are absent from populations 1, 2 and 4.

Analysis of variance of 18 characters (Table 5) indicated that there were seven characters showing variation significant at the $p<0.001$ level, two characters significant at the $p<0.001$ level, five characters significant at the $p<0.05$ level, and three characters not significantly variable.

Partitioning of the roots in the discriminant matrix of 18 measurements gave a total variance of 1588.636 (Table 6). Chi-square testing of each root shows that all roots are significant at the $p<0.05$ level. The first three components are highly significant at the $p<0.001$ level and explain 99.17% of the variance.

The projections of population means onto the first three discriminant functions resulted in three apparent groups of populations (Fig. 1). The first group consists of population 1, 2 and 4 and the second and third groups consist of population 3 and 5 respectively. A matrix of generalized distance between each pair of populations is shown in Table 7. The lowest

Table 4. The mean of characters.

Character	Population				
	1	2	3	4	5
1	88.567	81.900	92.800	62.400	15.263
2	0.800	0.733	0.900	2.067	0.250
3	0.233	0.167	0.300	0.600	0.250
4	5.567	5.533	6.500	4.500	3.250
5	—	—	0.100	2.733	—
6	36.870	32.358	29.312	33.290	4.110
7	17.502	17.630	16.947	15.503	27.707
8	1.515	1.274	1.903	1.532	0.900
9	2.057	2.016	1.387	2.678	1.513
10	2.644	2.278	3.153	2.544	0.878
11	17.450	14.350	15.110	23.500	18.025
12	1.307	1.153	1.810	1.877	1.320
13	9.250	7.250	9.200	15.117	12.375
14	1.197	1.040	1.610	1.787	1.325
15	2.015	1.922	2.459	2.309	2.415
16	1.075	0.977	1.030	1.257	1.017
17	0.463	0.420	0.467	0.587	0.390
18	—	—	0.300	13.533	18.750
19	—	—	—	44.933	61.290
20	4.683	3.833	2.300	6.367	6.250
21	—	—	—	32.667	35.000
22	1.833	0.633	1.400	17.133	14.00

Table 5. F-test of the variance among populations compared to the variance within population for 18 characters. The degrees of freedom are 4 and 99 respectively.

Character	MS_B	MS_W	F	Character	MS_B	MS_W	F
1.	7215.191	457.82	15.759***	11.	349.693	141.79	2.466*
2.	9.483	4.86	1.951	12.	2.503	0.51	4.908**
3.	0.825	1.495	0.552	13.	250.521	84.235	2.974*
4.	13.479	3.615	3.729**	14.	2.490	0.57	4.368**
6.	979.379	157.485	6.219***	15.	1.016	0.19	5.347***
7.	133.968	39.69	3.375*	16.	0.289	0.05	5.780**
8.	1.125	0.32	3.515**	17.	0.122	0.015	8.133***
9.	4.15	1.395	2.975*	20.	43.193	50.465	0.855
10.	4.237	0.63	6.725***	22.	1395.072	247.57	5.635***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 6. Test of significance of individual roots

DF	Root	X^2	D. F.	Critical value at $\alpha=0.05$
4	13.132	38.948	15	24.988
3	204.596	242.664	32	46.192
2	506.705	523.276	51	68.669
1	864.203 1588.636	850.957	72	92.810

DF=discriminant function; D. F.=degree of freedom

Table 7. Generalized distance matrix of five populations.

Population	1	2	3	4	5
1	0.				
2	1.719	0.			
3	5.853	5.937	0.		
4	4.655	5.318	7.640	0.	
5	13.704	12.526	12.203	14.384	0.

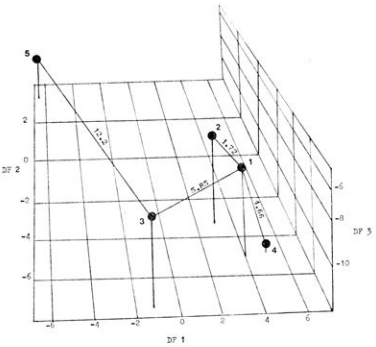

Fig. 1. Projection of means of five populations onto the first three discriminant functions based on 18 characters. The lines show the lowest value between pairs of populations in a generalized distance matrix. For explanation of population code number see Table 2. (DF=discriminant function).

generalized distance between a pair of populations is shown on the line linking them in Fig. 1. Populations, 1, 2 and 4 are closer to each other than to the other two populations. Population 3 is intermediate in position between the first group and population 5 on the first axis which explains 51.17% of the total variation in all populations.

Posterior classification of individuals following discriminant anelysis (Table 8) indicates most populations are morphologically very distinct. The only significant "error" in classification involves populations 1 and 2, these populations overlap each other morphologically to some degree. Specifically, nine individuals of population 1 fell into group B which is otherwise composed entirely of individuals from population 2. Five individuals of population 2 fell into group A. All individuals of population 3, 4 and 5 into group C, D and E respectively.

Table 8. The posterious classification of populations following discriminant function analysis. Rows correspond to populations and column correspond to discriminant groups.

Population	Discriminant groups				
	A	B	C	D	E
1	20	9	0	1	0
2	5	25	0	0	0
3	0	0	30	0	0
4	0	0	0	10	0
5	0	0	0	0	4

Thirty chromosome count from 14 natural populations in eastern Kansas were shown on Table 9. Among them 12 counts of four populations were studied phenetically by the discriminant function analysis in this paper.

DISCUSSION

Population 1, 2 and 4.

Population 1, 2 and 4 belong taxonomically to *Tradescantia ohiensis*. The chromosome counts of 68 populations of this species from central United States showed that 12 populations are diploid and 56 populations are tetraploid (Anderson, 1954; Anderson and Sax, 1936). The diploid populations are distributed in Texas, Louisiana, Michigan and Indiana, at the borders of this species. Populations 1, 2 and 4 of *T. ohiensis* from eastern Kansas have all been found to be tetraploid (Table 9).

Most species of the *virginiana* group are autopolyploid (Anderson and Diehl, 1932; Anderson and Woodson, 1935), it is possible that the diploid populations of *T. ohiensis* are remants of its diploid ancestor and from which tetraploid races were derived. Alternatively, introgressive hybridization from *T. ohiensis* into other diploid species could produce hybrids which are morphologically similar to the parent *T. ohiensis* but diploid. Such hybridization does occasionally occur in nature (Anderson and Hubricht, 1938; Riley, 1937, 1939). Still another other possibility is that *T. ohiensis* is primarily a tetraploid species which produces polyhaploid individuals, the diploid races. This apparently has occured rarely in other species (Kimber and Riley, 1963).

The generalized distance between population 1 and 2 is less than that between population

Table 9. Diploid chromosome numbers of *Tradescantia bracteata*, *T. ohiensis* and the putative hybrids collected from eastern Kansas.

Field No.	2n	Taxonomic species	Localities
568	24	*T. ohiensis*	Lyon Co.
569	24	*T. ohiensis*	Lyon Co.
570-2	12	*T. bracteata*	Greenwood Co.
570-3	12	*T. bracteata*	Greenwood Co.
570-4	12	*T. bracteata*	Greenwood Co.
571-2	24	*T. ohiensis*	Greenwood Co.
572-2	12	*T. bracteata*	Greenwood Co.
572-3	12	*T. bracteata*	Greenwood Co.
573-1	12	*T. bracteata*	Greenwood Co.
573-2	12	*T. bracteata*	Greenwood Co.
573-4	12	*T. bracteata*	Greenwood Co.
573-5	12	*T. bracteata*	Greenwood Co.
574-1	24	*T. ohiensis*	Butler Co.
575	24	*T. ohiensis*	Chase Co.
576-2	12	*T. bracteata*	Chase Co.
577-1	12	*T. bracteata*	Chase Co.
577-2	12	*T. bracteata*	Chase Co.
577-3	12	*T. bracteata*	Chase Co.
2017-1 (population 4)	24	*T. ohiensis*	Douglas Co.
2536-2 (population 3)	24	*T. bracteata*×*ohiensis*	Douglas Co.
2537-10(population 2)	24	*T. ohiensis*	Douglas Co.
2538-1 (population 1)	24	*T. ohiensis*	Douglas Co.
2538-3 (population 1)	24	*T. ohiensis*	Douglas Co.
2538-4 (population 1)	24	*T. ohiensis*	Douglas Co.
2538-4 (population 1)	24	*T. ohiensis*	Douglas Co.
2538-9 (population 1)	24	*T. ohiensis*	Douglas Co.
2538-19(population 1)	24	*T. ohiensis*	Douglas Co.
2538-29(population 1)	24	*T. ohiensis*	Douglas Co.
2540-1 (population 3)	24	*T. bracteata*×*ohiensis*	Douglas Co.
2540-2 (population 3)	24	*T. bracteata*×*ohiensis*	Douglas Co.

1 and 4 (Fig. 1), and this relationship parallels the similarity of the habitat which they occupied. The sites of populations 1 and 2 appear similar ecologically, both being roadside prairie with wind and traffic, and probably favor similar adaptive combination of genes. Population 1 and 2 have similar flowering period (Table 3). Population 4 grows in shade at the foot of the wooded hill.

The posterior classification (Table 7) showed that one individual from population 1 fell into population 4 which *T. ohiensis* forms a distinct group. The habitat of population 1 is more variable, and may have gene combinations which are adapted to a niche similar to that of population 4. Glandular hair is completely absent from population 1, 2 and 4. This

character was not utilized in the discriminant analysis in this study due to its zero variance in these three populations. The presence of glandular hairs is discontinuous between this group and *T. bracteata* populations and therefore, provides a good criterion for investigating hybridization between *T. ohiensis* and *T. bracteata*.

Population 5.

Population 5 taxonomically belongs to *T. bracteata* which is a diploid species (Anderson, 1954; Anderson and Sax, 1939). Discriminant analysis indicates that this population is morphologically very distinct even though its most distinctive feature (glandular pubescence) was not utilized in the analysis (Fig. 1).

Population 5 has been observed to start flowering in mid April and stop in May, spending the summer in a dormant condition (Table 3). The other four populations begin flowering in late April or early May and continue to July (Table 3). Thus a partial seasonal isolation restricts the gene exchange between population 5 and the others.

Sixteen chromosome counts from 8 populations of *T. bracteata* from eastern Kansas area are all diploid (Table 9). Hybridization between *T. bracteata* and *T. ohiensis* may occasionally occur in nature (Anderson and Hubricht, 1938) but chromosome counts of hybrid type plants rarely stated. It is evident that the triploid hybrids would have low fertility. The chromosome number difference presumably serves as a partial barrier between these two species.

These two species are also isolated by a different ecological preferences. *T. bracteata* grows best in a habitat of cool temperature and moist soil, *T. ohiensis* grows best in a habitat of higher temperature and drier soil.

Population 3.

In the three dimension model (Fig. 1) and posterior classification following discriminant analysis, population 3 is clearly distinct from the others. The generalized distance shows that population 3 is closer phenetically to the first group (population 1, 2 and 4) than to population 5. Population 3 scores high for number of branches, total number of branch internodes, length of sheath, and size of floral bracts and sepals (Table 4). However, for other characters, including the abundance of glandular hairs, population 3 is intermediate between population 5 and the group composed of population 1, 2 and 4. Chromosomally population 3 being tetraploid is similar to the first group. But glandular hairs are a specific character of *T. bracteata* and completely absent from *T. ohiensis;* the glandularly hairy population 3 can not be a variable form of *T. ohiensis*. Clearly the vigorous external feature, intermediate characters, and tetraploid chromosome provide evidence that population 3 could be derived from hybridization between *T. bracteata* and *T. ohiensis*. However, it bears glandular hairs on pedicel and sepal and following the taxonomical criterion of Anderson and Woodson (1935) it would apparently fall into *T. bracteata*. Thus it is possible that the tetraploid *T. bracteata* populations described by Anderson could be the result of introgressive hybridization from *T. bracteata* to *T. ohiensis*.

Two possible sequences of chromosomal changes are proposed to account for the putative hybrid population 3 which shows definite morphological similarities to the diploid *T. bracteata* and also the tetraploid *T. ohiensis*. One possible derivation (Fig. 2) starts with a triploid hybrid between tetraploid *T. ohiensis* and diploid *T. bracteata*. The triploid ($2n=18$) hybrid then produces gametes with chromosome number ranging from six to 12 and a gamete with 12 chromosome united with a gamete from *T. ohiensis* producing a tetraploid backcross hybrid. The tetraploid hybrid repeatedly backcrossing with *T. ohiensis* could produce the putative hybrid derivative population 3. Alternatively, a diploid *T. bracteata* could produce an autotetraploid to cross with a tetraploid *T. ohiensis* producing a tetraploid F_1 hybrid. Repeated back crosses with *T. ohiensis* could produce the putative hybrid population 3 (Fig. 3).

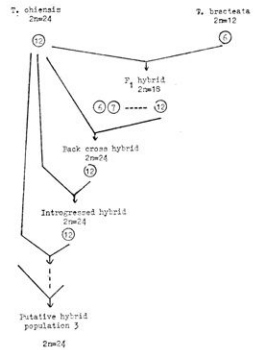

Fig. 2. Hypothetic trend (a) of chromosomal evolution in the putative hybrid population 3. Circles represent gametes.

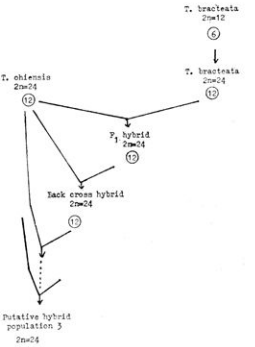

Fig. 3. Hypothetic trend (b) of chromosomal evolution in the putative hybrid population 3. Circles represent gametes.

Population 3 grows along a railroad. The construction of a railroad created an open habitat. This provided a chance for the first hybrid generation to grow due to the relaxation of competition and selection pressure (Grant, 1971). The first hybrid generation is often highly sterility and provides less viable gametes than the parental species. There is a much more chance for the hybrid gametes to be united with the parental gametes than with each other. This leads to the formation of back cross hybrids (Stebbins, 1950). Since *T. ohiensis* (very long flowering period) provides more chance to cross with the F_1 hybrids than *T. bracteata* (short flowering period) does, the second back cross generation predominantly consists of individuals whose genotype approach to *T. ohiensis*. The back cross progeny is more likely to back cross again because of their ecological preference close to one of the parental species (Anderson and Hubricht, 1938). Repeat back crossing generation after generation established population 3.

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