INVESTIGATION ON THE POPULATION VARIATION OF *Drosera indica* L. COMPLEX USING COMBINED MORPHOLOGICAL AND MOLECULAR TECHNIQUES

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ABSTRACT

Drosera indica L. is an annual, tropical species of carnivorous plant exhibiting a considerable amount of morphological variability, including plant size, flower colour, stamen form, seed size, and seed coat ornamentation pattern. Thus far there has been no study dealing with these morphological variability. The present study, therefore, is aiming at investigating the pattern of morphological and genetic variability in this species to determine whether there are morphologically distinguishable groups, and whether these groups are genetically distinct.

Materials used in this study consisted of air-dried herbarium specimens, water- and silica sand-preserved plant, and glasshouse- and tissue culture-grown plants germinated from seeds. The assessment of morphological variation was carried out on sixty two accessions of D. indica based on 62 accessions based on 14 vegetative and floral characters, as well as 12 micromorphological seed characters examined using scanning electron microscope. Multivariate numerical analysis on morphological data was performed using cluster analysis and two ordination techniques: the multidimensional scaling and principal component analysis. The pattern of genetic variation was evaluated on 15 accessions of D. indica using random amplified polymorphic DNA (RAPD). The DNA for RAPD analysis was obtained from fresh materials only, either from glasshouse- or tissue culture-grown plants germinated from seeds. The other types of materials failed to produce DNA of sufficient amounts and quality.

Results of morphological data analysis indicated that there are six morphotypes, each representing a distinctive combination of seed type and other morphological characters. Examination on the geographic distribution of accessions, coupled with the geology and the average annual rainfall data suggested that these morphotypes occurred sympatrically, and that they did not exhibit distinct geographical and ecological patterns. Based on this

evidence, therefore, these morphotypes might represent varieties within *D. indica*, or possibly even distinct species.

Cluster analysis and multidimensional scaling ordination on RAPD data revealed a high degree of genetic dissimilarity between accessions and between different morphotypes. The grouping of accessions based on RAPD data did not correspond to that resulted from morphological analysis. A comparison on the same set of samples (15 accessions) indicated that accessions from different morphotypes grouped together in the same cluster generated from RAPD data, and that there was no consistent pattern in the grouping of these morphotypes. This result indicated that there were differences in the pattern of within-species morphological and genetic variation. The discrepancy between results from morphological and molecular data was discussed. The two data sets, however, are in general agreement in detecting the degree of similarity between accessions.

The high degree of genetic dissimilarity revealed from RAPD analysis confirms the inbreeding nature of D. indica, and provides evidence on the reproductive isolation between sympatric morphotypes. This result, therefore, supports the recognition of the six defined morphotypes as distinct species. Considering the wide range of distribution of D. indica across different habitats and continents, however, further examination of specimens covering as much as possible its range of geographic distribution and morphological variation is required to justify the suggested taxonomic treatment.

DECLARATION

This work contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institutions and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Ratna Susandarini

21/6/2001

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TABLE OF CONTENTS

ABSTRACT	i
DECLARATION	iii
ACKNOWLEDGMENTS	iv
Table of Contents	v
List of Tables	viii
List of Figures	ix
Chapter One: Introduction	1
Chapter Two: Literature Review	4
2.1 The Genus <i>Drosera</i>	4
$2.1.1\ Drosera\ indica\ { m L}.$	7
2.2 DNA-based Molecular Markers in Plant Systematics	10
2.3 Polymerase Chain Reaction (PCR)	13
2.4 Random Amplified Polymorphic DNA (RAPD)	16
2.4.1 RAPD in Infraspecific Study of Plants	20
2.4.1.1 The Use of RAPD in the Estimation of Genetic Diversity	21
2.4.1.2 The Use of RAPD in the Identification of Cultivars and Species	23
2.4.2 Analysis of RAPD Data in Systematics Studies	25
2.5 The Use of Herbarium Specimens for DNA-based Studies in Plant Systematics	29
2.6 Morphological and Molecular Data in Systematics Studies	31
Chapter Three: Seed Morphology of Drosera indica L.	36
3.1 Abstract	36
3.2 Introduction	36
3.3 Materials and Methods	41

3.3.1 Materials	41
3.3.2 Methods	42
3.4 Results	43
3.5 Discussion	48
3.6 Conclusion	53
Chapter Four: DNA Extraction Methods for <i>Drosera indica</i> L.	54
4.1 Abstract	54
4.2 Introduction	54
4.3 Materials and Methods	58
4.3.1 Plant Material	58
4.3.2 Sample Preparation	60
4.3.3 DNA Extraction Protocols	60
4.3.4 Spectrophotometry	65
4.3.5 Gel Electrophoresis of DNA	65
4.4 Results	66
4.5 Discussion	68
4.6 Conclusion	73
Chapter Five: Morphological Variation in the <i>Drosera indica</i> Complex	74
5.1 Abstract	74
5.2 Introduction	74
5.2.1 Morphology in Taxonomy	75
5.2.2 Infraspecific Variation and Classification in Plant	76
5.2.3 Phenetic Analysis in Taxonomy	79
5.2.3.1 Cluster Analysis	81
5.2.3.2 Ordination	82
5.2.4 Studies in Species Complexes Using Phenetic Methods	84
5.3 Materials and Methods	85
5.4 Results	88
5.4.1 Cluster Analysis	88
5.4.2 Multidimensional Scaling Analysis	91
5.4.3 Principal Component Analysis	93

5.5 Dis	scussion	96
5.6 Co	nclusion	104
Chapter Si	x: Morphological and Genetic Variation in the Drosera indica Complex	105
6.1 Ab	stract	105
6.2 Int	roduction	105
6.5	2.1 Molecular data in taxonomy	106
6.2	2.2. Species concepts in plant systematic study	107
6.3 Ma	aterials and Methods	109
6.3	3.1 Morphological analysis	109
6.3	3.2 RAPD analysis	110
$6.4~\mathrm{Re}$	sults	112
6.4	4.1 Morphological analysis	112
6.4	4.2 RAPD analysis	114
6.5 Dis	scussion	117
6.6 Co	nclusion	122
Chapter Se	even: General Discussion	123
REFEREN	CES	131
APPENDI	CES	
I.	Accessions used in this study	160
II.	Results of examination on seed morphological characters	162
III	Herbarium specimens of <i>Drosera indica</i> L. showing variation in morphology (plant size, stem colour, and flower colour)	166
IV.	Scores and measurements of morphological data	167
V.	Glasshouse-grown plant of <i>Drosera indica</i> showing a red stripe on the abaxial surface of the leaf	173
VI.	The Geology of Western Australia	174
VII.	Map of the Average Annual Rainfall of Western Australia	175
VIII.	Gel electrophoresis of RAPD fragments of <i>Drosera indica</i> amplified using primer OPA03	176

LIST OF TABLES

Table 3.1	Seed morphological characters examined	39
Table 3.2	Grouping of seed samples based on seed coat ornamentation patterns	41
Table 4.1	List of materials used in DNA extraction study	59
Table 4.2	Comparisons of DNA yield and quality from accessions extracted using four different protocols	67
Table 5.1	Morphological characters examined	86
Table 5.2	Coefficient loadings of characters in the first three components of PCA	94
Table 5.3	Geographic position where different morphotypes were found growing together in a mixed population in Kimberley region, Western Australia	101
Table 6.1	Accessions of <i>Drosera indica</i> used in morphological and RAPD analysis	110

LIST OF FIGURES

Figure 3.1	Scanning electron micrograph of <i>Drosera indica</i> seeds with reticulate ornamentation pattern (Type I), showing three different seed coat cell shapes: a. tetragonal (AL2242); b. transversely hexagonal (AL2219); c. longitudinally hexagonal (AL1208).	42
Figure 3.2	Scanning electron micrograph of <i>Drosera indica</i> seeds with (a) foveolate (Type II) ornamentation (AL1306), and (b) longitudinally ridged or furrowed (Type III) ornamentation (AL1730).	43
Figure 3.3	Scanning electron micrograph of epicuticular wax deposits on the surface of the seed coat, showing: a. irregular granules; b. rosettes; c. rounded granules. All forms are from seeds within the same sample (Dro42).	44
Figure 4.1	Gel electrophoresis of DNA showing comparison of DNA integrity obtained from different materials	67
Figure 5.1	UPGMA dendrogram of 62 accessions of <i>Drosera indica</i> based on morphological data, showing six morphological groups	90
Figure 5.2	The MDS analysis of <i>Drosera indica</i> accessions using Gower's metric, showing the groups from the dendrogram in Figure 5.1	92
Figure 5.3	Two-dimensional scatter-plot of <i>Drosera indica</i> accessions projected in PC1 and PC2, showing six morphological groups and outliers	95
Figure 5.4	Distribution map of some <i>Drosera indica</i> accessions in Kimberley region, Western Australia	100
Figure 6.1	UPGMA dendrogram of 15 accessions of <i>Drosera indica</i> based on 16 morphological characters, showing the grouping of accessions into four groups	113
Figure 6.2	The MDS analysis of 15 accessions of <i>Drosera indica</i> based on 16 morphological characters, showing the grouping of accessions into four groups	114

Figure 6.3	UPGMA dendrogram of 15 accessions of <i>Drosera indica</i> based on RAPD data	116
Figure 6.4	The MDS analysis of 15 accessions of <i>Drosera indica</i> based on RAPD data	117

R. SUSANDARINI ERRATA: page/paragraph/line

3/2/4-5 'D. ramentacea Burch, ex DC., D.	102/1/3 'with a distinctive'
madagascariensis DC., D. burmanni Vahl and	102/1/4 delete 'and thus'
D. peltata Thunb. is recognised as the'	102/1/8 'population systems'
7/2/1 'areas, and' = 'areas, that'	102/1/17 'in the case of D. indica'
7/2/10 'Asia to' = 'Asia, Japan'	103/2/1 'that of the six defined morphotypes, 3 (A,
9/3/1 remove 'L.'	B and C)
9/3/2 remove 'a set of characters such as'	104/1/2 delete 'six defined'
10/2 remove all 'L.'	104/2/1 'comprised three morphotypes and a
11/2/1 'resulted in a new'	further three subtypes'
12/1/1 replace 'amount' with 'number'	105/1/10 'inbreeding'
13/2/5 'of a specific'	105/1/11 ' the three morphotypes'
13/2/6 'by a factor'	106/2/7 'similarity and difference'
15/2/8 'researchers: Welsh'	108/1/1 replace 'in which the' with 'so that a'
18/1/5 'from the heterozygote	108/2/1 'replace' from with 'of'
18/3/2 replace 'overcome' with 'reduced'	108/2/6 'which is a commonly used'
19/1/9 'in conjunction with'	108/2/9 'reproductive'
23/2/5 'kinds'	109/1/1 'fingerprinting technique using RAPD'
31/2/11 'data may have'	110/1/2 delete 'random amplified polymorphic
35/1/8 remove 'each'	DNA'
35/2/1 remove 'research'	112/2/2 'B2 and C, corresponding to the main
35/2/2 'variability between specimens'	clusters identified in the earlier morphological
36/1/6 replace 'Provided' with 'Combined';	analysis'
'evident' with 'evidence'	112/2/3 'Cluster A (seed Type II)
38/2/4 'either help to define'	112/2/5 'Cluster B1 (seed Type I)
38/2/14 'At the infraspecific level'	112/2/7 'Cluster B2 (seed Type I)
39/1/10 'species on seed morphology'	112/2/9 'Cluster C (seed Type III)
40/1/5 delete 'genus'	112/2/10 'and red-striped petiolate leaves'
40/1/9 delete 'was'	117/1/3 delete 'result'
40/2/7 delete 'the family'	118/1/5 '(Abbott et al. 1985)'
40/2/9 'as in the study'	119/2/2 replace 'inbreeding' with 'inbred'
49/1/3 'SEO meant that'	119/2/3 'that is responsible'
51/1/4 'excavations'	119/2/5 replace 'explaining' with 'explains'
52/2/2 'Type II seeds'	119/2/10 'populations from exchanging genes'
52/3/4 'belonging to'	120/2/10 'Whitkus, 1997), whereas'
53/1/2-3 'which no additional character was found	121/2/2 'populations'
to support the'	122/1/2 'difference, and'
55/1/2 replace 'quality' with 'concentration'	122/1/8 'as much as'
55/2/5 delete 'cell'	122/1/9 'conclusion could'
66/3/6 'sources'	124/2/8 replace 'defining' with 'studying'
69/2/11 delete 'because'	126/1/1 delete 'are'
69/2/12 'hydration, so the'	127/3/2 'cases where a pair'
69/3/1 delete 'using'	128/1/14 'is the nature of morphology'
69/3/4 delete 'from'	128/1/21 'and that with a large'
76/3/title 'plants'	128/1/22 'there is a possibility that'
77/3/4 '1986;'	129/3/2 replace ', thus confirms its inbreeding
79/2/5 'thus does not necessarily'	nature' with 'providing evidence of
80/2/8 'character overlap with'	reproductive isolation between accessions,
81/2/4 'replace 'to use' with 'that'	possibly through inbreeding'
81/2/5 'analysis to be used in'	130/2/3 'distinct species. The anecdotal pollination
84/1/3 delete 'in the application of numerical	observations where there is clear pollinator
phenetic methods'	preference between the different morphs also
84/2/12 delete 'two'	supports the idea of genetic isolation. However,
85/2/3 'fifty-nine complete specimens'	considering'
88/2/1 'dendrogram'	130/2/5 'as possible of its range'
91/1/2 'of the clusters (Fig. 5.4)'	100,200 as possible of its fairgo
91/4/1 replace 'Despite' with 'In addition to'	



Drosera indica L.