

## SYSTEMATIC IMPLICATIONS OF ISOZYME NUMBER VARIATION IN TRIBE BRASSICEAE (CRUCIFERAE)

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## ABSTRACT

Tribe Brassiceae (54 genera) is generally recognized as one of the few natural (monophyletic) groups in the Cruciferae. The placement of *Calepina*, *Conringia* and *Orychophragmus* in the tribe has been questioned, as has the validity of the seven subtribes. The present study examined isozyme number, which is usually highly conserved in diploid plants, in order to assess the evolution of chromosome number and systematic relationships in the tribe. Twelve enzyme systems were surveyed for 102 Brassiceae species in 34 genera. Isozyme numbers in taxa with  $n \leq 13$  and  $n = 14-18$  were similar. Both groups exhibited the minimum isozyme number expected for diploid plants for the majority of isozymes surveyed. Duplications were observed for several isozymes for both groups of taxa, suggesting a widespread occurrence in the tribe of a secondarily-balanced polyploid genome structure. The observed variation in isozyme number among taxa was also phylogenetically informative. Duplications for cytosolic PGM and TPI supported the monophyletic nature of the tribe, with the exclusion of *Calepina* and *Conringia*, and the inclusion of *Orychophragmus*. The *Fbp-2* duplication supported a monophyletic origin of *Brassica rapa* and the *B. oleracea* cytodeme.

## INTRODUCTION

Tribe Brassiceae, widely regarded as one of the few natural groups in the Cruciferae (Al-Shehbaz, 1985), is subdivided into seven subtribes (Schulz, 1936) and includes some 54 genera and 219 species. Recent molecular analyses, however, have questioned the validity of this subtribal classification and have proposed new generic and subtribal circumscriptions (Warwick and Black, 1993; in press). The placement of *Calepina*, *Conringia* and *Orychophragmus* in the tribe has also been questioned (Al-Shehbaz, 1985). Within the tribe, chromosome numbers vary from  $n = 6-75$  (Warwick and Anderson, 1993). Taxa with  $n > 13$  are believed to be polyploid (Al-Shehbaz, 1985), although there is no evidence to support this claim.

Isozyme number is usually highly conserved in diploid plants and increases in this number (=isozyme duplications) are believed to be rare events and therefore of phylogenetic significance (Gottlieb, 1982). Past studies (Arús *et al.*, 1991; Chen *et al.*, 1990) have suggested that isozyme number variation might represent a useful data set for investigating phylogenetic relationships in the tribe Brassiceae. The objectives of the following study were: 1) to assess ploidy level in the tribe, 2) to identify isozyme duplications, and 3) to test phylogenetic relationships using these duplications.

## METHODS

In total, 102 species and 34 genera from tribe Brassiceae and 11 species from 7 additional tribes were examined (Table 1). Within the Brassiceae, a range of chromosome numbers ( $n = 7-60$ ) was represented. Horizontal starch gel electrophoresis was used to determine isozyme number for 11 isozymes: *Adh-1*, *Fba-1*, *Fbp-1*, *Fbp-2*, *Gpi-1*, *Gpi-2*, *Idh-2*, *Me-1*, *Pgm-1*, *Pgm-2*, *Tpi-1*. By comparing 5-10 individuals from each accession, it was possible to distinguish between multiple bands representing genetic variation occurring at a single locus and those due to isozyme duplications.

TABLE 1. Taxa surveyed including 34 genera (no. of species) of the Brassiceae and 11 species from other tribes

<u>Tribe Brassiceae</u>		
<i>Brassica</i> (24)	<i>Erucaria</i> (5)	<i>Psychine</i> (1)
<i>Cakile</i> (2)	<i>Erucastrum</i> (9)	<i>Raffenaldia</i> (1)
<i>Calepina</i> (1)	<i>Euzomodendron</i> (1)	<i>Raphanus</i> (2)
<i>Carrichtera</i> (1)	<i>Fortuynia</i> (1)	<i>Rapistrum</i> (2)
<i>Ceratocnemum</i> (1)	<i>Guiraoa</i> (1)	<i>Rytidocarpus</i> (1)
<i>Coincya</i> (2)	<i>Hirschfeldia</i> (1)	<i>Schouwia</i> (1)
<i>Conringia</i> (2)	<i>Kremeriella</i> (1)	<i>Sinapidendron</i> (2)
<i>Crambe</i> (9)	<i>Moricandia</i> (5)	<i>Sinapis</i> (4)
<i>Diplotaxis</i> (10)	<i>Orychophragmus</i> (1)	<i>Succowia</i> (1)
<i>Eranthrocarpus</i> (1)	<i>Pseuderucaria</i> (1)	<i>Trachystoma</i> (3)
<i>Eruca</i> (1)	<i>Pseudofortuynia</i> (1)	<i>Vella</i> (2)
		<i>Zilla</i> (1)
<u>Outgroup taxa</u>		
<i>Alyssum dasycarpum</i>	<i>Erysimum cheiranthoides</i>	<i>Sisymbrium irio</i>
<i>Arabis caucasica</i>	<i>Isatis tinctoria</i>	<i>Taucheria lasiocarpa</i>
<i>Barbarea vulgaris</i>	<i>Lepidium sativum</i>	<i>Thlaspi arvense</i>
<i>Berteroa incana</i>	<i>Lunaria annua</i>	

## RESULTS and DISCUSSION

Ploidy level

Isozyme numbers in taxa with  $n \leq 13$  and taxa with  $n = 14-18$  (*Crambe*, *Euzomodendron*, *Fortuynia*, *Moricandia*, *Pseuderucaria*, *Psychine*, *Rytidocarpus*, *Schouwia*, *Succowia*, *Vella* and *Zilla*) were similar. Both groups exhibited the minimum isozyme number expected for diploid plants for the majority of isozymes surveyed. Duplications were, however, observed for several isozymes (*Fbp-1*, *Fbp-2*, *Gpi-2*, *Idh-2*, *Me-1*, *Pgm-2*, *Tpi-1*). These data suggested a widespread occurrence in the tribe of a secondarily-balanced polyploid genome structure consistent with that originally proposed for the three *Brassica* crop species (reviewed in Prakash and Hinata 1980). The data also suggested that aneuploidy has played a more significant role in the evolution of base chromosome numbers among taxa in the tribe than polyploidy.

Phylogenetic relationships

The isozyme duplications were phylogenetically informative at various taxonomic levels. Only duplications for *Tpi-1*, *Pgm-2* and *Fbp-2* are reported here. *Tpi-1* and *Pgm-2* duplications support the monophyletic nature of the tribe with the inclusion of *Orychophragmus* and the exclusion of *Calepina* and *Conringia*.

The *Tpi-1* duplication was observed in 28 of the 34 genera examined in tribe Brassiceae. The duplication was not observed in any of the outgroup taxa and would appear to be diagnostic for the tribe, supporting the inclusion of *Orychophragmus*. The lack of detection of the duplication in *Carrichtera*, *Enarthrocarpus*, *Euzomodendron* and *Succowia* was likely due to fixation of the same allele at both loci as there was evidence of the duplication in closely allied taxa. It was not possible to extrapolate the same assumption for *Calepina* and *Conringia* since they lack close relatives in the tribe. The absence of the *Tpi-1* duplication from these taxa supports their exclusion from the tribe.

The *Pgm-2* duplication was observed in all taxa examined in tribe Brassiceae with the exception of *Calepina* and *Conringia*, again supporting their exclusion from the tribe. The *Pgm-2* duplication was also detected in several outgroup taxa (*Alyssum*, *Barbarea*, *Erysimum*, *Isatis*, *Lepidium*, *Lunaria*). The *Pgm-2* duplication may also be a useful taxonomic tool for determining relationships among tribes in the Cruciferae.

The *Fbp-2* duplication was observed in five species of *Brassica* and one species of *Diplotaxis*, supporting a monophyletic origin for *B. rapa* and four members (*B. cretica*, *B. insularis*, *B. macrocarpa* and *B. villosa*) of the *B. oleracea* cytodeme, consistent with other data sets (Warwick and Black, 1993). Given the morphological and molecular distinctness of *Diplotaxis viminea* (Warwick and Black, 1993), it is likely that its duplication arose independently.

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