

## Genotypic variation for floral characters in *Brassica* and allied genera with special reference to breeding system

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The five floral characters, i.e., number of pollen grains per flower, pollen grain diameter, anther length, number of ovules per flower and pollen-ovule ratio (P/O), were studied on 119 strains of 53 species in *Brassica* and allied genera with respect to the breeding system. There were large variations in all five characters among species, especially the number of pollen grains, number of ovules per flower and P/Os showed larger variations. The values range widely from 23–24 × 10<sup>4</sup> of *Eruca* spp. to 0.3 × 10<sup>4</sup> of *Diplotaxis viminea* and from 19800 of *Hutera rupestris* to 100 of *D. viminea* in the number of pollen grains and P/Os, respectively. The P/O was significantly correlated to the energy cost per flower. Both indices were closely related with the breeding system of species. The lowest P/Os and the smallest energy costs were found in the obligate autogamous species, while the xenogamous species showed higher and larger, but more fluctuating P/Os and energy costs. In the facultative autogamous amphidiploid species in *Brassica* crops, both P/Os and energy costs per flower were similar to those of xenogamous species; however, P/Os were lower than their xenogamous parental species. The relationships of P/Os with natural selection are also discussed.

**Key Words:** *Brassicinae*, *Brassica*, breeding system, floral variation, pollen-ovule ratio (P/O).

### Introduction

The variations of overall floral shape and floral components represent adaptations to various modes of pollination. Many reviews have reported the characteristics of floral morphology associated with breeding systems.

Ornduff (1969) listed 32 floral characters that distinguished xenogamous plants from their autogamous derivatives for the cruciferous genus *Laevenworthia*. He indicated that the switch from xenogamy to autogamy had been accompanied by alterations in many morphological characters, mostly floral. Cruden (1977) mentioned that decreasing flower size and alterations in floral morphology, which mediated the evolutionary shift in the breeding system, reduced the energy cost per flower and facilitated self-pollination. Further, he emphasized that pollen-ovule ratios (P/Os) were a better predictor of a plant's breeding system than other morphological characters. Later, Spira (1980), studying the California species of *Trichostema* (Labiatae), reported that three floral parameters, flower size, pollen-ovule ratio (P/O) and nectar volume, were correlated with the breeding system (autogamy vs. xenogamy) and pollinator type (bee vs. bird). The data showed that xenogamous plants expended more energy per floral unit and were less efficient seed producers than closely related autogamous species.

The species classified in subtribe *Brassicinae* hold our attention, because these members are related to crop Brassicas and are potential genetic resources for improving these crops. Although the floral structures of these species are composed of 1 pistil, 6 stamens, 4 petals and 4 sepals, several kinds of breeding system exist in this group. Hinata and Nishio (1980) reported that in 59 species in the *Brassicinae*, 50 species are self-incompatible and 9 are self-compatible. On the other hand, most previous studies that have dealt with floral diversity in members of the Cruciferae have chiefly centered on their use in taxonomic treatment and/or diagnostic descriptions of the species (Horovitz and Cohen 1972, Clemente and Hernandez-Bermejo 1980, Gómez-Campo 1980) and on intraspecific variation of floral traits for the production of F<sub>1</sub> hybrid seeds (Pierre *et al.* 1999, Yoshioka *et al.* 2005, Syafaruddin *et al.* 2006). Little has been reported on the relationships between variations of floral characters and the breeding system. The exception is the study by Hinata and Konno (1975), in which the definite relationships between the number of pollen grains per flower and the breeding system in *Brassica* and allied genera were given. In a study based on observations of P/Os of 66 crucifer taxa in California, Preston (1986) reported that P/Os can serve as a standard for indicating the breeding system.

It is reported that P/Os are also related with plant habitats and/or successional stages involving adaptation strategies (Cruden 1977, Ramirez and Seres 1994, Chouteau *et al.* 2006). In particular, Cruden (1977), after studying more than 80 different species, indicated that P/Os increase significantly

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from disturbed habitats to late successional seres.

The object of the present study was to evaluate the variation of floral characters in the subtribe *Brassicinae* and to clarify the relationships of P/Os with energy costs per flower, breeding systems and adaptation strategies.

## Materials and Methods

### Plant materials

The materials used were composed of 119 strains of 53 species in subtribe *Brassicinae*: 48 strains of 18 species in *Brassica*, 31 strains of 13 species in *Diplotaxis*, 4 strains of 2 species in *Eruca*, 16 strains of 8 species in *Erucastrum*, 5 strains of 1 species in *Hirschfeldia*, 7 strains of 6 species in *Hutera* (syn. *Coincya*), 1 strains of 1 species in *Sinapidendron*, and 7 strains of 4 species in *Sinapis* (Table 1). They were preserved in the Plant Breeding Lab., Tohoku University, Japan. Details of these strains, including growing conditions, have been mentioned in previous papers (Takahata and Hinata 1980, 1986). Plants were grown in pots of 24 cm diameter in a glass house.

### Investigation of floral characteristics

The five floral characters, number of pollen grains per flower, pollen grain diameter, anther length, number of ovules per flower and P/O, were investigated. The number of pollen grains per flower was determined using a hemacytometer according to Hinata and Konno (1975). Flower buds one day before anthesis were collected randomly during the middle of flowering and fixed in 3 : 1 ethanol-acetic acid. All anthers removed from a flower bud were placed in a test tube, with 0.3 ml solution of 50% glycerin water containing 0.08% cotton blue. The solution with anthers was stirred by a 2 cm magnet. After checking to see that no pollen grains remained in the anthers, the number of pollen grains in the test tube was estimated by a hemacytometer. The pollen grain diameter was measured at the same time, and was presented as the mean value of 20–40 pollen grain samples from each of four plants that were randomly collected in each strain. The anther length was measured using anthers on long stamens. The number of pollen grains per flower and the anther length was presented by the average of four plants and five flowers were taken for each plant. The number of both placentas in the valve and seeds in the beak, which were already reported in the previous paper (Takahata and Hinata 1980), were summed up in substitution for the number of ovules per flower.

Principal component analysis and other statistical analyses were carried out utilizing STATPAC (NEC) and/or JMP (SAS Institute Inc.).

## Results

The value of the five floral characters, number of pollen grains per flower, pollen grain diameter, anther length, number of ovules per flower and P/Os, of each strains of 53

species in *Brassicinae* are given in Table 1. There are large variations in all five characters among species, especially the number of pollen grains, number of ovules per flower, and P/Os showed larger variations. The number of pollen grains per flower ranged widely from 23–24 × 10<sup>4</sup> of *Eruca* to 0.3 × 10<sup>4</sup> of *D. viminea*. *B. oleracea* (17–23 × 10<sup>4</sup>) and *Hutera* spp. (12–21 × 10<sup>4</sup>) showed a large number of pollen grains, while *B. tournefortii*, *D. muralis* and *Er. abyssinicum* had a small number of pollen grains (less than 2 × 10<sup>4</sup>). The number of ovules per flower varied from 8–9 of *B. souliei* and *B. nigra* to 96–130 of *D. harra*. The values of P/Os also varied widely from 19800 of *Hu. rupestris* to 100 of *D. viminea*.

Table 2 shows correlation coefficients between the five characters. The number of pollen grains per flower showed the highest positive correlation with anther length ( $r = 0.708$ ), and the highest negative correlation with pollen diameter ( $r = -0.548$ ). There was a positive correlation between P/Os and the number of pollen grains per flower ( $r = 0.585$ ) and a negative correlation between P/Os and the number of ovules per flower ( $r = -0.426$ ).

Principal component analysis was carried out on six floral characters, i.e., petal length and width, pistil length, sepal length, anther length and number of pollen grains per flower. Of these, data of four characters, petal length and width, pistil length and sepal length, were taken from previous reports (Takahata and Hinata 1980, 1986). Eighty-five percent of total variation was extracted by the first component. The first component score expressed the variation of the general size factor of characters, and it was assumed that this score reflected the energy cost per flower. Figure 1 is a scatter diagram of strains on the two characters of P/O and the first component score, showing that these characters are closely correlated ( $r = 0.578$ ,  $P < 0.001$ ). This means that plants with high P/O have a larger energy cost per flower, while those expending only small energy for floral units have lower P/Os.

Obligate autogamous species, such as *B. tournefortii* (B51–B53), *D. viminea* (D32) and *Er. abyssinicum* (M01, M02), had the lowest P/Os and the smallest flowers. *D. muralis* (D15, D16) and *Er. gallicum* (M06), which are autogamous allotetraploid, show the lowest P/Os but slightly larger flowers (energy cost per flower) than the obligate species. Either or both of the genomes comprising these two species originated from xenogamous parental species (Harberd 1976, Takahata and Hinata 1983), seemingly reflected in the slightly larger costs per flower. The P/Os of autogamous *B. oxyrrhina* (B06–B08) were not greatly reduced, but the energy cost per flower was smaller than most xenogamous *Brassica* species.

Three amphidiploid species of *Brassica* crops, *B. carinata*, *B. juncea* and *B. napus*, are self-compatible and reported to be only partially outcrossing (Rives 1957, Olsson and Persson 1958), and are regarded as facultative autogamous, even though all the genomes are from xenogamous parental species. Both the P/O and energy cost per flower of these three *Brassica* crop species, *B. carinata* (B13, B14),

**Table 1.** Five floral characters of 119 strains of 53 species in subtribe *Brassicinae*

Species	Strain	Code no.*	ANL (mm)	POD ( $\mu$ m)	NPG	NOV	P/O
<i>B. souliei</i>	B01	Am-3	1.20	50	27489	8.8	3124
	B02	Am-4	1.21	50	19509	8.2	2379
<i>B. barrelieri</i>	B03	Ba-103	2.40	50	73224	25.3	2894
	B04	Ba-106	2.43	52	70385	29.0	2427
	B05	Ba109	2.33	50	78310	19.6	4016
<i>B. oxyrrhina</i> <sup>+</sup>	B06	Ox-101	1.67	50	35031	16.1	2189
	B07	Ox-107	1.63	50	40605	14.9	2725
	B08	Ox-108	1.58	42	35031	15.2	2305
<i>B. rapa</i> ssp. <i>toria</i>	B09	C-504	2.58		96445	16.8	5741
	B10	C-506	2.78		111803	19.3	5793
<i>B. rapa</i> ssp. <i>chinensis</i>	B11	C-333	3.19		128925	23.7	5440
<i>B. rapa</i> ssp. <i>campestris</i>	B12	C-479	2.38	50	113129	24.8	4562
<i>B. carinata</i> <sup>+</sup>	B13	Ca-101	3.26		97864	16.2	6041
	B14	Ca-104	2.92		66003	21.0	3143
	B15	Ca-112	3.39		84308	19.2	4391
<i>B. deflexa</i>	B16	Df-1	2.58		136188	33.8	4029
<i>B. elongata</i>	B18	El-102	2.40	40	88036	19.9	4424
<i>B. fruticulosa</i> ssp. <i>fruticulosa</i>	B19	Fr-103	2.01	60	40145	17.0	2361
	B20	Fr-104	1.69	60	29780	18.8	1584
	B21	Fr-503	2.08	50	55707	41.4	1346
	B23	Fr-202	2.01	58	48669	21.0	2318
	B22	Fr-201	2.11		42582	43.8	972
<i>B. fruticulosa</i> ssp. <i>cossoniana</i>	B24	Fr-301	3.07	60	64559	24.8	2605
<i>B. fruticulosa</i> ssp. <i>radicata</i>	B25	Fr-502	2.34	60	38651	20.0	1933
	B26	Fr-401	2.83	60	51891	20.3	2556
<i>B. fruticulosa</i> ssp. <i>mauritanica</i>	B27	Gr-1	2.09	50	56967	38.6	1476
<i>B. gravinae</i>	B28	Gr-2	3.17		77578	39.3	1974
	B30	J-113	2.58	52	53327	17.0	3137
<i>B. mauroum</i>	B33	Ma-1	2.30		63085	24.1	2618
	B34	Ma-2	2.11		56982	30.8	1850
	B35	Ma-5	2.23		60849	27.8	2189
<i>B. napus</i> <sup>+</sup>	B36	N-101	2.98		92819	25.4	3654
	B37	N-132	2.97		72472	25.8	2809
	B38	N-137	3.23		83369	24.7	3375
	B39	N-472	2.84		94042	21.1	4457
<i>B. nigra</i>	B40	Ni-116	1.88	44	50019	8.8	5684
	B41	Ni-138	2.15	50	62299	7.6	8197
	B42	Ni-141	2.45	50	77607	9.3	8345
<i>B. oleracea</i> ssp. <i>acephala</i>	B43	O-166	3.95		225116	29.5	7631
	B44	O-169	4.12		234091	34.3	6825
<i>B. oleracea</i> ssp. <i>capitata</i>	B46	O-8	3.99		166766	32.3	5163
<i>B. repanda</i> ssp. <i>maritima</i>	B47	Re-4	3.39	60	70180	36.9	1902
<i>B. repanda</i> ssp. <i>nudicaulis</i>	B48	Re-5	2.66	60	52207	31.7	1647
<i>B. spinescens</i>	B50	Sp-1	2.20	60	50408	22.4	2250
<i>B. tournefortii</i> <sup>+</sup>	B51	T-165	1.41	50	19423	18.3	1061
	B52	T-167	1.33	50	17772	21.5	827
	B53	T-162	1.14		12128	20.4	595
<i>B. desnottesii</i>	B54	Ds-1	2.19	60	47561	20.3	2343
<i>D. assurgens</i>	D01	ASS-1	1.91		57253	27.9	2052
	D02	ASS-2	1.58		59013	34.4	1720
	D03	ASS-3	1.53		56037	32.0	1751
<i>D. berthautii</i>	D04	BER-1	1.84		45688	46.1	991
<i>D. catholica</i>	D05	CAT-4	1.62		30307	44.8	676
	D06	CAT-5	1.72	60	39239	44.4	884

Table 1. (continued)

Species	Strain	Code no.*	ANL (mm)	POD ( $\mu$ m)	NPG	NOV	P/O
<i>D. eruroides</i>	D07	ERU-4	2.51		128994	46.9	2750
	D08	ERU-7	2.41		107951	57.6	1874
	D09	ERU-9	2.51		115304	42.9	2688
<i>D. harra</i> ssp. <i>harra</i>	D10	HAR-1	2.76		127775	130.1	982
	D11	HAR-4	3.26	50	137076	108.5	1263
<i>D. harra</i> ssp. <i>crassifolia</i>	D12	HAR-6	2.68	46	129213	124.1	1041
<i>D. harra</i> ssp. <i>lagascana</i>	D13	HAR-8	2.81	50	104545	98.2	1065
	D14	HAR-9	2.94	48	159521	95.8	1665
<i>D. muralis</i> <sup>+</sup>	D15	MUR-1	2.29	78	20675	58.2	355
	D16	MUR-3	2.20		16896	51.8	326
<i>D. pitardiana</i>	D17	PIT-1	2.45		63132	104.7	603
<i>D. siifolia</i>	D18	SII-2	1.94	50	53689	29.3	1832
	D19	SII-3	2.14	52	68878	21.6	3189
	D20	SII-4	2.08		46398	31.4	1482
<i>D. tenuifolia</i>	D21	TEN-3	2.76	50	81671	55.7	1466
<i>D. tenuisiliqua</i>	D22	TSQ-1	1.51	40	52155	16.0	3260
	D24	TSQ-6	1.58	42	45409	17.0	2671
	D25	TSQ-7	1.39		44398	18.3	2426
	D26	TSQ9	1.57	46	41140	17.6	2338
	D27	VIR-4	2.06	50	62834	77.2	814
<i>D. virgata</i>	D28	VIR-10	1.96	56	62716	89.8	698
	D29	VIR-12	1.67		45922	56.6	811
	D30	VIR-13	1.76		38056	55.3	688
<i>D. siettiana</i>	D31	SIE-1	2.05	50	54931	83.5	658
<i>D. viminea</i> <sup>+</sup>	D32	VIM-1	0.63	58	3398	34.3	99
<i>E. sativa</i>	E01	SAT-9	3.37		232182	19.6	11846
	E02	SAT-12	3.29		243793	26.6	9165
<i>E. vesicaria</i> ssp. <i>vesicaria</i>	E03	VES-3	3.22		230331	26.3	8758
	E04	VES-5	3.39	40	238124	31.4	7584
<i>Er. abyssinicum</i> <sup>+</sup>	M01	ABY-1	1.44	60	12613	44.0	287
	M02	ABY-2	1.67	56	12189	51.7	236
<i>Er. arabicum</i>	M03	ARA-1	2.60	60	57048	24.0	2377
<i>Er. cardaminoides</i>	M05	CAR-1	1.81		32050	17.0	1885
<i>Er. gallicum</i> <sup>+</sup>	M06	GAR-1	2.06		30241	36.7	824
<i>Er. leucanthum</i>	M08	LEU-1	2.15	40	113752	48.8	2331
<i>Er. nasturtiifolium</i>	M09	NAS-1	2.68	40	152829	40.5	3774
	M10	NAS-2	2.62	40	141862	44.6	3181
	M11	NAS-3	2.54	40	122819	42.1	2917
	M12	VAR-2	1.94	60	45929	24.4	1882
	M13	VAR-3	1.90	50	60746	26.8	2267
<i>Er. varium</i>	M14	VAR-6	1.54	40	48618	21.9	2220
	M15	VAR-7	1.64	44	48617	24.8	1960
	M16	VAR-8	1.90	44	70993	32.0	2219
	M17	VAR-10	1.86	54	38188	24.7	1546
<i>Er. elatum</i>	M20	ELA-1	1.94	50	37713	17.6	2143
<i>Hi. incana</i> ssp. <i>incana</i>	HI1	Ad-111	1.48	50	37206	8.8	4228
	HI2	Ad-112	1.44		41747	9.0	4639
	HI5	Ad-119	1.64	48	43807	9.1	4814
<i>Hi. incana</i> ssp. <i>consobrina</i>	HI3	Ad-115	1.78		50661	8.8	5757
<i>Hi. incana</i> ssp. <i>geniculata</i>	HI4	Ad-116	1.47	46	39882	9.9	4028
<i>Hu. granatensis</i>	H02	GRA-1	2.40	40	177670	39.7	4475
<i>Hu. hispida</i>	H04	HIS-2	2.52	40	193749	32.1	6036
<i>Hu. leptocarpa</i>	H05	LEP-1	2.13	40	174125	11.6	15011
<i>Hu. longirostra</i>	H06	LON-1	2.39		171086	22.3	7672

**Table 1.** (continued)

Species	Strain	Code no.*	ANL (mm)	POD ( $\mu\text{m}$ )	NPG	NOV	P/O
	H07	LON-2	2.00		120854	23.1	5232
<i>Hu. pseudoerucastrum</i>	H08	PSE-2	2.99	40	211742	29.0	7301
<i>Hu. rupestris</i>	H09	RUT-1	2.56	40	200478	10.1	19849
<i>Sd. rupestre</i>	SD2	SDRUP-1	2.02		59626	38.8	1537
<i>S. alba</i>	S01	ALB-25	2.87	60	53905	4.9	11001
	S02	ALB-28	2.62	60	65227	5.5	11859
<i>S. arvensis</i>	S03	ARV-13	2.21	60	51366	10.0	5137
	S04	ARV-16	2.21	60	47342	11.5	4117
	S05	ARV-18	2.36		45527	10.8	4215
<i>S. pubescens</i>	S07	PUB-1	3.39	60	43351	15.6	2779
<i>S. turgida</i>	S08	TUR-1	2.89		64003	8.9	7191

\* Code no. of Tohoku University *Brassica* seed bank ([http://www.agri.tohoku.ac.jp/pbreed/Seed\\_Stock\\_DB/SeedStock-top.html](http://www.agri.tohoku.ac.jp/pbreed/Seed_Stock_DB/SeedStock-top.html)).

+ Autogamous species

ANL: Anther length, POD: Pollen grain diameter, NPG: No. of pollen grains per flower, NOV: No. of ovules per flower, P/O: Pollen-ovule ratio

**Table 2.** Correlation coefficients among 5 floral characters in 119 strains of 53 species in subtribe *Brassicinae*

	ANL	NPG	POD	NOV
NPG	0.708			
POD	0.034	-0.548		
NOV	0.179	0.180	-0.012	
P/O	0.388	0.585	-0.284	-0.426

ANL: Anther length, NPG: No. of pollen grains per flower, POD: Pollen diameter, NOV: No. of ovules per flower, P/O: Pollen-ovule ratio. Correlation coefficients of POD with other characters were based on 72 strains of 38 species (see Table 1)

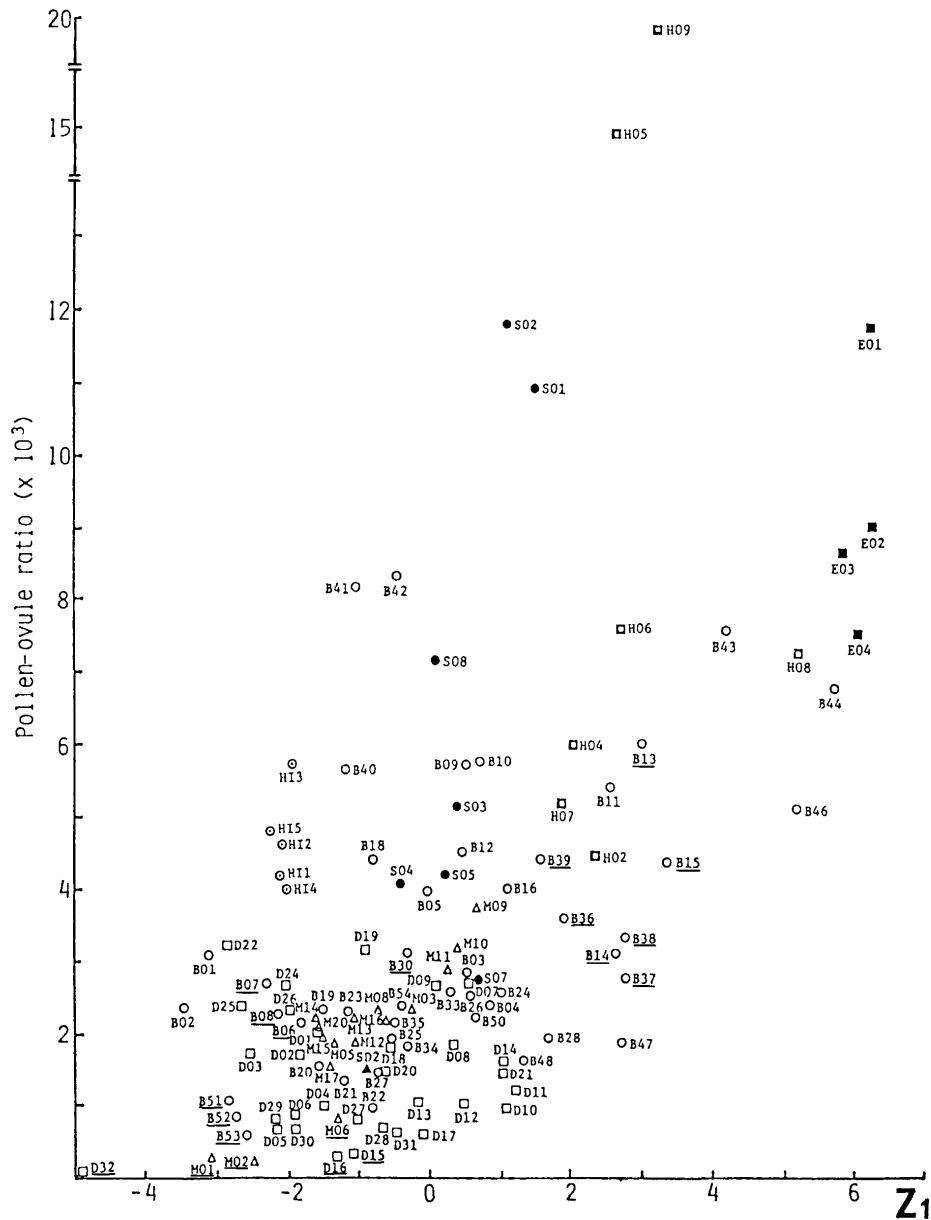
*B. juncea* (B30) and *B. napus* (B36–B39), were very difficult to distinguish from those of xenogamous species in *Brassica* (Fig. 1); however, when these amphidiploid species are compared with their parental species, it was revealed that they show intermediate energy cost per flower between their xenogamous parental species and lower P/Os than their parents, except for B13 strain of *B. carinata* (Fig. 2). These results seem to suggest that these facultative autogamous amphidiploids have obtained a more efficient device for pollination than xenogamous parental species, although no reduction in energy cost per flower occurred.

Among xenogamous species of *Brassicinae*, the P/O and energy cost per flower varied widely (Fig. 1). On the whole, the variation of these two indices was quite continuous among species and genera; however, each genus seems to have a characteristic distribution; for example, *Eruca* and *Hutera* species had the high P/Os and the large energy cost per flower. *Eruca* species had the largest energy per flower, while *Hu. rupestre* (H09) and *Hu. leptocarpa* (H05) had the highest P/Os. Among *Sinapis* species, in spite of the rather constant energy cost per flower, P/Os ranged widely from 11900 of *S. alba* (S02) to 2800 of *S. pubescens* (S07). *Hi. incana* (HI1–HI5) had relatively high P/Os (4000–5800). Most *Brassica* species are xenogamous and are distributed widely in the diagram. The P/O value of *B. fruticulosa* ssp.

*cossoniana* (B22) was very similar to that of autogamous species, while *B. nigra* (B40–B42) and *B. oleracea* (B43, B44, B46) were closer to *Eruca* species. Concerning the energy cost per flower, *B. soulieri* (B01, B02) was very similar to autogamous species, while *B. oleracea* was close to *Eruca* and *Hutera* species. *B. nigra* showed the highest P/O among *Brassica* species, notwithstanding its relatively small flowers. A similar case was also seen in *S. alba* (S01, S02). The distribution of *Diplotaxis*, *Erucastrum*, and *Sinapidendron* species overlapped that of *Brassica* in the diagram (Fig. 1). In *Diplotaxis*, however, *D. harra* cytodeme (D10–D14), *D. tenuifolia* cytodeme (D17, D21) and *D. sittiana* (D31) increased the energy cost per flower without an accompanying distinct increase in P/Os.

## Discussion

In general, our results indicated that the shift from xenogamy to autogamy is accompanied by reduced floral size and decreased P/Os. This agrees with the results of previous research, as described in Ornduff (1969), Cruden (1977), Spira (1980), and Preston (1986). Of nine autogamous species, three obligate species, *B. tournefortii*, *D. viminea* and *Er. abyssinicum*, had the smallest energy cost per flower and the lowest P/Os. The three amphidiploid species of *Brassica* crops, *B. carinata*, *B. juncea* and *B. napus*, showed intermediate energy cost per flower between their xenogamous parental species and lower P/Os than their parent. The fact that these facultative autogamous *Brassica* crops have maintained the higher energy cost per flower and remnants of the parental outcrossing mechanism (Ohsawa and Namai 1987) suggests their recent derivation from xenogamous species. Two autogamous allotetraploids, *D. muralis* and *Er. gallicum*, which show very low P/Os, almost identical to those of obligate autogamous species, but with slightly larger energy cost per flower, have retained a partial outcrossing mechanism by means of their attractive larger flowers. The autogamous diploid, *B. oxyrrhina*, was characteristic of



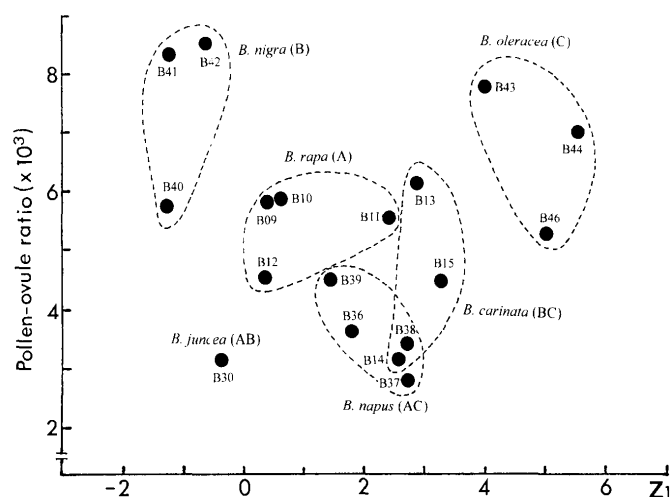
**Fig. 1.** 119 strains scattered according to the pollen-ovule ratio and the first component scores ( $Z_1$ ) by principal component analysis based on six floral characters, petal length, petal width, pistil length, sepal length, anther length and number of pollen grains per flower. The first component score could be considered as an indicator of energetic cost per flower, because this expressed the variation of general size factor of flower. Strains underlined are autogamous species. ○: *Brassica*, □: *Diplotaxis*, ■: *Eruca*, △: *Erucastrum*, ⊙: *Hirschfeldia*, ◻: *Huttera*, ▲: *Sinapidendron*, ●: *Sinapis*.

a much reduced energy cost per flower in spite of rather high P/Os. This seems to imply that *B. oxyrrhina* is in the process of changing from facultative to obligate autogamy. As with flower size, flower number is known as another important character for pollination (Conner and Rush 1996). Some xenogamous species, such as *B. souliei* (B01, B02), have similar flower size as obligate autogamy, but show higher P/Os. For such species, the flower number is considered to be important for pollinator attraction; however, since flower number was not investigated in this study, these points remain to be elucidated in the future.

Molecular phylogenetic studies (Warwick *et al.* 1992, Ohshima *et al.* 2001) revealed two distinct lineages, Rapa/Oleracea and Nigra, in subtribe *Brassicinae*. Autogamous

species present in both lineages, for example, *B. oxyrrhina*, *D. viminea* and *Er. abyssinicum*, belong to Rapa/Oleracea, and *B. tournefortii* to Nigra, indicating that the change in breeding systems occurs independently of species differentiation.

Cruden and Miller-Ward (1981) proposed and tested the hypothesis that P/O is inversely related to (1) the likelihood of pollen grains reaching a stigma and (2) the pollen size in xenogamous plants. In the present study, a negative relationship between P/O and pollen grain size, even though not very significant statistically, was also found ( $r = -0.284$ ). In addition, it became clear that P/Os were closely related to energy cost per flower ( $r = 0.578$ ,  $P < 0.001$ ). Similar cases have been found in the relationships between flower diameter



**Fig. 2.** Three autogamous amphidiploid species and their xenogamous parental species in *Brassica* crops scattered according to the pollen-ovule ratio and first component scores ( $Z_1$ ) as described in Fig. 1.

( ): Genome symbol.

and P/O in *Trichostema* of Labitatae (Spira 1980). Although *Brassicinae* species showed positive relationships between flower size and P/O value, in species such as *B. nigra*, *S. alba* and *Hi. incana*, high P/Os were not always accompanied by large floral energetics (Fig. 1 and Fig. 2). This is due to incomplete estimation of energy cost per flower. In this study, the indicators of the energy cost per flower were estimated without determining nectar production; therefore, the energy cost per flower of these species may have been underestimated.

*Brassicinae* species do not only have dissimilar habitats but also have very different lifestyle forms, consisting of many annuals or biennials and a few perennials (Tsunoda 1980). Some species of *Diptotaxis* have as low P/Os as obligate autogamous plants in spite of their large floral energetics and grow in an unstable environment or disturbed habitat. *D. harra* plants, for instance, grow in the desert, being exposed to seasonal aridity. Such species can be regarded as ruderal (R) selected plants defined by Grime (1977). Most species with higher P/Os, on the other hand, are plants with secondary strategies. *Hutera* species grow on rocky outcrops in mountainous regions and *B. oleracea* on coastal cliffs that are always exposed to salinity. Since these locations would be interpreted as slightly disturbed unproductive habitats, they may be regarded as competitive (C)–stress-tolerant (S) strategists. *S. alba* grows in regions with abundant moisture and fertile soil such as foothills and the edges of ditches. *B. nigra* is usually found on slopes between roads and crop fields. Such species seem to have adapted to environments with low stress impact and with the competition restricted to moderate intensity by disturbance. These are viewed as C-R-plants. *Eruca* species are commonly found in unfertilized meadows. As the competition is restricted to moderate intensity by the combined effects of stress and disturbance in such habitats, they are regarded as C-R-S-plants. From the above

examples, it is concluded that, in the subtribe *Brassicinae* as a whole, species with low P/Os are R-selected plants and with increased P/Os, they seem to shift from R- to C- or/and S-selected plants.

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