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Artificial selection on trichome number in *Brassica rapa*

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Summary. We examined genetic variation for trichome production in a rapid-cycling population of *Brassica rapa* by conducting a selection experiment and by growing progeny from maternal seed families. Data from the maternal families were also used to estimate the genetic correlations between trichome number and (1) number of days to first flower and (2) flower production. For seven generations, 10% of the plants were selected from low, high and control lines with 100 individuals per line per generation. The number of trichomes on the right edge of the first leaf was 20.8 ± 13.4 (mean + SD; $n = 100$) in the base population, and had by the final generation reached 93.9 ± 28.7 ($n = 100$) in the high line and 0.9 ± 2.6 ($n = 100$) in the low line. Control line plants of the seventh generation did not differ significantly from base population plants in number of trichomes on the edge. The realized heritability of trichome number on the edge was 0.38 (based on the regression of cumulative response on cumulative selection differential). The divergence between lines in trichome production on the edge of the first leaf was associated with a divergence in trichome number on the petiole and on the top of the same leaf, and on the edge, top and petiole of the third leaf. The increase in trichome production in the high line was further associated with a significant delay in flowering time relative to the control and low lines. The estimated heritabilities of trichome number on the edge and the petiole of the first leaf and of days to first flower were not significantly different from 1.0 (based on the among maternal family component of the total variance). Trichome number on the edge showed a significant genetic correlation with trichome number on the petiole of the first leaf ($r = 0.80$), and with number of days to first flower ($r = 0.31$), but not with total flower production ($r = 0.17$; $n = 83$). The substantial genetic variation for trichome production in *B. rapa* is of potential value for breeding purposes and also makes *B. rapa* a convenient system for the study of plant-herbivore interactions.

Key words: *Brassica rapa* – Genetic correlations – Heritability – Selection experiment – Trichome production

Introduction

Trichome production has been found to be negatively correlated with the damage caused by a diverse array of insect herbivores in several important crop species (Levin 1973; Norris and Kogan 1980; Stipanovic 1983; Southwood 1986). Trichomes may interfere with insect feeding and oviposition and have in some cases been noted to impale soft-bodied athropods.

Studies of factors determining oviposition and feeding by the major herbivores on *Brassica* spp. have focused on the chemical composition of the plants (e.g., Feeny 1977; Chew 1988; Renwick 1988; Lamb 1989). However, in addition to a variation in the production of secondary compounds and leaf waxes, there is also substantial inter- and intraspecific variation in trichome production in *Brassica* (Gómez-Campo 1980; Nishi 1980). Lamb (1980) showed that trichomes on the pods of *Brassica hirta* deter feeding of the flea beetle *Phyllotreta cruciferae* (Goeze), and we have recently found that larvae of *Pieris rapae* (L.) (Lepidoptera: Pieridae) preferentially feed on *B. rapa* with low trichome production (Ågren and Schemske unpublished).

The potential for natural or artificial selection to change trichome number is determined by the amount of

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additive genetic variation available and also by the genetic and phenotypic correlations between trichome number and components of fitness such as viability and fecundity. To study the genetic basis of trichome number in *Brassica rapa*, we performed a selection experiment, and we grew maternal seed families obtained through controlled crosses. Our objectives were to estimate the heritability of trichome number and to estimate the genetic correlations between trichome production and (1) number of days to first flower and (2) total flower production.

Material and methods

Brassica rapa L. (syn. *campestris*; Brassicaceae), rape or field mustard, is a self-incompatible annual or biennial with significant agricultural importance, and it is, in addition, a cosmopolitan weed. In this study we used a rapid-cycling stock of *B. rapa* obtained from the Crucifer Genetics Cooperative at the University of Wisconsin (*Brassica* CRCG stock #1, Aaa, Williams 1985). The rapid-cycling line of *B. rapa* was originally established by combinations of various early-flowering types, followed by several cycles of selection for early flowering, rapid seed maturation, absence of seed dormancy, small plant size and high female fertility (Williams and Hill 1986). *B. rapa* produces setiform trichomes on leaves and stems. The leaf trichomes are 0.6±0.10 mm long (mean±SD, $n=30$ [5 trichomes measured on each of 6 plants of the rapid-cycling line]).

To obtain a large base population for our experiments, the original seed lot was bulked by random outcrossing of 100 plants in the greenhouse. We performed selection on the number of trichomes on the right edge of the first true leaf. Initially, 100 plants belonging to the base population were scored for trichome number on the right edge, on the right half of the top and on the petiole of the first true leaf. These three characters were significantly intercorrelated (edge versus top $r=0.57$, $P=0.0001$; edge versus petiole $r=0.62$, $P=0.001$), and we chose to use the number of trichomes on the edge since this was the character that was the easiest to score. The plants were ranked according to trichome number on the edge, and the top 10, the bottom 10 and a random sample of 10 plants were selected as parents for the first generation of the high, low and control lines, respectively. In each of the six subsequent generations, trichome number on the right edge of the first true leaf was scored on 100 plants per line (10 plants from each maternal parent). Within each line plants were ranked according to trichome number on the edge, and the top 10% in the high line, the bottom 10% in the low line and a random 10% of the plants in the control line were chosen as parents for the next generation. To examine how trichome production on the petiole responded to selection on trichome number on the edge, all plants were scored for number of trichomes on the petiole of the first true leaf.

To determine if selection on trichome number resulted in a change in leaf size, we measured the length of the first leaf on all low and high line plants for the first three generations. Finding no significant differences in leaf length between the high and low lines in any of these generations (first generation $t=1.78$, $P=0.08$; second generation $t=1.05$, $P=0.29$; third generation $t=0.82$, $P=0.41$; df$=198$), we discontinued the leaf length measurements.

The selected plants were pollinated to produce at least eight fruits each. Each flower received pollen from one randomly assigned pollen donor from within the selected group. At pollination, newly dehisced anthers were brushed across the stigma until it was densely covered with pollen.

The selection experiment was continued for seven generations. To document any changes in number of days to first flower, the seventh generation was grown together with a set of plants from the base population ($n=108$), and the first day of flowering was noted for all plants. To quantify any divergence in trichome production on other parts of the plant, trichomes were counted in additional positions on the first (right half of the top) and the third leaf (right edge, right half of the top, and petiole) on 30 plants/line in the seventh generation.

To estimate the phenotypic correlations between trichome production, number of days to first flower and fecundity, the 108 base population plants grown together with the seventh generation were also scored for number of trichomes on the edge and on the petiole of the first leaf, and cumulative flower number by day 52 (at senescence).

Maternal seed families were obtained by performing crosses on 83 plants belonging to the base population. The plants were pollinated for 10 days. Each day 4 plants were randomly assigned pollen donors for each maternal plant. Pollen from these 4 plants was mixed and applied to all available stigmas of the maternal plant. We expect the pollination procedure to have resulted in maternal families consisting mainly of half-sibs. Five seeds per maternal plant were subsequently planted, and the following characters were scored: trichome number on the right edge and on the petiole of the first true leaf, number of days to first flower and cumulative flower number by day 52.

The selection experiment was carried out in a growth room at 20–24 °C and under fluorescent light 24 h/day. The maternal seed families were, because of space limitation, grown in a greenhouse having supplemental lighting 12 h/day. A preliminary study showed that the mean and variance in trichome number on the edge did not differ between base population plants grown in the growth room (20.0±13.0, mean±SD; $n=108$) and those grown in the greenhouse (19.0±14.4; $n=100$), suggesting that trichome production was not affected by the differences in the growth conditions between the two environments. Furthermore, in both environments most plants were senescing after 7 weeks of growth. All plants were grown in 7.5×7.5×5.5 cm square pots and were fertilized with a weak solution of complete fertilizer at 10 days and subsequently every week throughout flowering and fruit maturation. In the selection experiment watering was discontinued 20 days after the last pollination had been performed, and the plants were allowed to dry for 10 days before the seeds were harvested.

The realized heritability of trichome number on the edge of the first leaf was estimated from the regression of cumulative response on cumulative selection differential, where cumulative response is defined as the cumulative divergence in trichome number between the high and low lines (Hill 1972). From the family data, the heritabilities (h^2) of trichome number on the edge and the petiole, of days to first flower and of total flower production were estimated as

$$h^2=4\ V_f/V_t$$

where V_f is the among maternal family variance component, and V_t is the total phenotypic variance (Falconer 1989). This measure will overestimate the true heritability if families are partly composed of full-sibs and should therefore be regarded as an upper limit for the true value. The estimate may be further inflated by maternal effects, since it is based on maternal sibships. Standard errors and confidence limits on heritability estimates were calculated following Becker (1984). Genetic correlations between trichome number, number of days to first flower and total flower number were estimated as the Pearson correlation coefficients using maternal family means (see Via 1984). Confidence limits

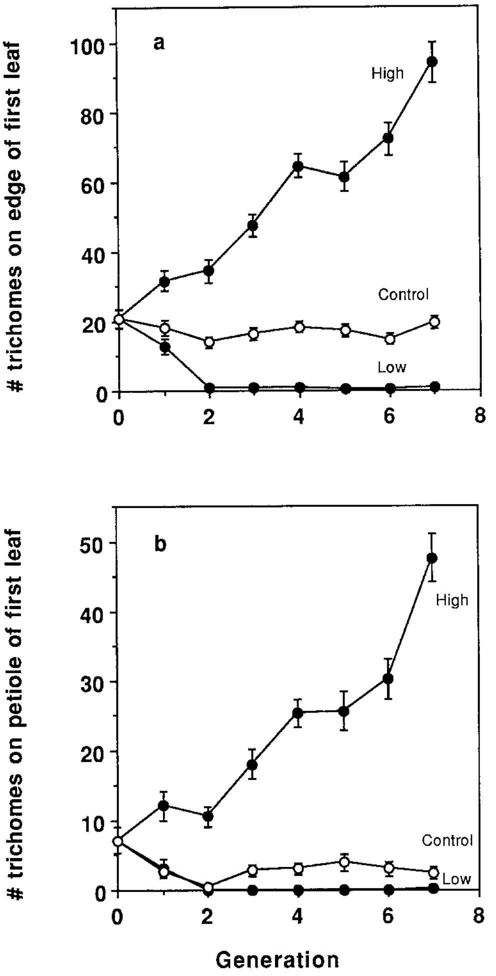

Fig. 1a, b. Number of trichomes on the right edge (**a**) and the petiole (**b**) of the first true leaf (Means ± 2 SE; $n = 100$) in the *Brassica rapa* selection experiment. Selection was performed on the number of trichomes on the edge of the first true leaf

to the genetic correlation coefficients were set by means of the z-transformation (Sokal and Rohlf 1981). When appropriate, data were log-transformed to conform better to assumptions of normality.

Results

Directional selection for high and low trichome number on the edge of the first true leaf was effective in causing a divergence in trichome production between lines (Figs. 1 and 2). The realized heritability of trichome number on the edge of the first leaf was 0.38. In the high line the average number of trichomes on the right edge of the first leaf had increased by more than five standard deviations by the seventh generation, from 20.8 ± 13.4 (mean ± SD; $n = 100$) in the base population to 93.9 ± 28.7. In the low line the average trichome production on the edge had dropped to 1.1 ± 2.4 by the second generation and was less than 1 in all subsequent generations. In the seventh generation, 73% ($n = 100$) of the plants in the low line did not produce any trichomes on the leaf edge. Control line plants of the final generation did not differ from the base population in number of trichomes on the edge ($t = 0.9$, NS; $df = 198$)

The divergence in trichome production on the leaf edge was associated with a divergence in trichome number in other regions of the plant. The number of trichomes on the petiole of the first leaf was 7.3 ± 9.8 (mean ± SD; $n = 100$) in the base population. By the seventh generation it had increased to 47.6 ± 16.8 in the high line and decreased to 0.2 ± 1.3 in the low line (Fig. 1b). The lines showed also a distinct divergence in number of trichomes on the top of the first leaf and on various parts of the third leaf (Table 1).

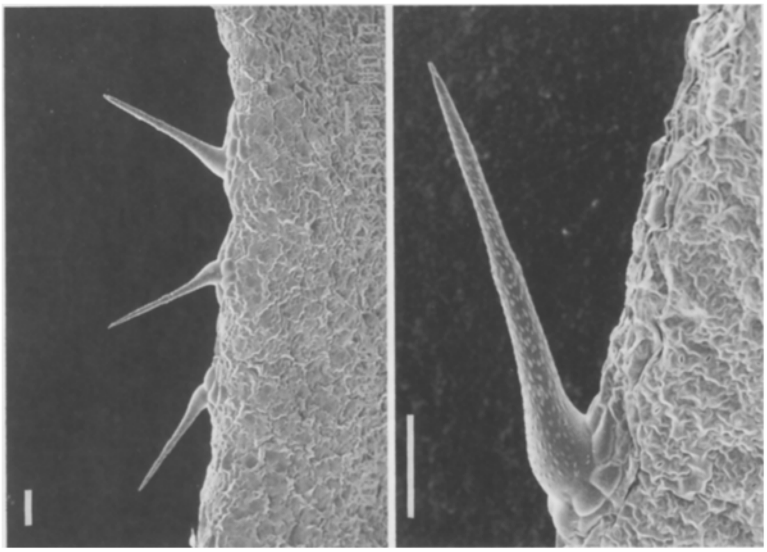

Fig. 2. Scanning electron micrographs of trichomes on the edge of the first true leaf of a plant belonging to the seventh generation of the line selected for high trichome number. *Bars:* 100 µm

Table 1. Number of trichomes on the first and third true leaves, and number of days to first flower (means±SD) in the base population and in the selected lines of *Brassica rapa* after seven generations of selection performed on trichome number on the edge of the first leaf. Top refers to number of trichomes on the right half of the top; edge to number of trichomes on the right edge of the leaf

	Base	Low	Control	High	*n*
First leaf					
Top	4.5±7.86[a]	0.1±0.37	1.6± 4.58	46.8±18.44	30
Third leaf					
Edge	N.D.	0.3±0.84	15.5±15.40	174.3±46.66	30
Top	N.D.	0 ±0	1.0± 4.48	48.3±31.46	30
Petiole	N.D.	0.1±0.73	0.5± 1.91	23.8±13.74	30
Days to first flower	17.5±1.69[b]	19.7±1.65	19.5± 1.68	21.0±2.19	100

N.D., No data available

[a] $n = 100$

[b] $n = 108$

Table 2. Variance in trichome number on the right edge and on the petiole of the first true leaf, in days to first flower, and in total flower production in a rapid-cycling population of *Brassica rapa*. ANOVA's were performed on log-transformed data obtained from maternal seed families

Source of variation	*df*	Trichome number on the edge		Trichome number on the petiole		Days to first flower		Total flower number	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Among families	82	1.307	3.66***	3.751	3.63***	0.0341	3.70***	0.560	2.58***
Within families	332	0.357		1.032		0.0092		0.217	

*** $P < 0.001$

Table 3. Variance components for trichome number, flowering start, and total flower number in a rapid-cycling population of *Brassica rapa*, as estimated from 83 maternal seed families

Source of variation	Trichome number on the edge		Trichome number on the petiole		Days to first flower		Total flower number	
	Variance component	Percent	Variance component	Percent	Variance component	Percent	Variance component	Percent
Among families	0.190	34.7	0.544	34.5	0.00498	35.1	0.0686	24.0
Within families	0.357	65.3	1.032	65.5	0.00921	64.9	0.2170	76.0
Total	0.547	100.0	1.576	100.0	0.01419	100.0	0.2856	100.0

The increase in trichome production in the high line was associated with a small, but significant, delay in flowering. On average, high line plants began flowering 1.5 days later than control line plants and 1.3 days later than low line plants, in the seventh generation ($F_{2,292} = 19.8$; $P = 0.0001$; Table 1). All selected lines began flowering later than the base population (Table 1).

We observed significant among-family variation for trichome number, flowering start and total flower number (Tables 2 and 3). The estimated heritability of trichome number on the edge of the first leaf was 1.39 ± 0.22 ($h^2 \pm$SE), of trichome number on the petiole 1.38 ± 0.22, of days to first flower 1.40 ± 0.22, and of total flower production 0.96 ± 0.21 (all significantly different from zero, $P < 0.05$; heritability estimates based on the among-family variance components given in Table 3). The estimated heritabilities of these characters were all very high, although they did not differ significantly from the theoretical maximum of 1.0 ($P > 0.05$). There was a strong positive genetic correlation between trichome number on the edge and on the petiole of the first leaf (Table 4). Trichome production on the edge was also significantly (positively) correlated with number of days to first flower, but not with total flower number (Table

Table 4. Phenotypic and genetic correlations in the base population of *Brassica rapa*. Genetic correlations were calculated from maternal family means and are presented with 95% confidence intervals in parentheses. All data were log-transformed prior to analysis

	Phenotypic correlations ($n=108$)	P	Genetic correlations ($n=83$)	P
Trichome number on edge × Trichome number on petiole	0.67	<0.01	0.80 (0.71–0.87)	<0.01
Trichome number on edge × Days to first flower	0.31	<0.01	0.31 (0.10–0.49)	<0.01
Trichome number on edge × Total flower number	−0.07	N.S.	0.17 (−0.05–0.37)	N.S.
Days to first flower × Total flower number	0.19	<0.05	0.46 (0.27–0.61)	<0.01

4). There was a significant positive genetic correlation between days to first flower and total flower production. In the base population the phenotypic and genetic correlations were qualitatively similar: in three cases both correlations were significant and positive; in the remaining case they were both insignificant (Table 4).

Discussion

This study has demonstrated that there is substantial genetic variation for trichome number in rapid-cycling *Brassica rapa*. There was a continuous response to selection for increased trichome production, and there was no indication that a plateau had been reached after seven generations of selection. These observations suggest that trichome number is inherited as a quantitative character in this species. In *Arabidopsis thaliana* four non-allelic mutants that show variously strong reductions in trichome number have been identified (Koornneef et al. 1982; Marks and Feldmann 1989), indicating that trichome number is a character influenced by several genes in that species also.

The realized heritability of trichome production on the edge of the first leaf was 0.38. This was markedly lower than the estimated heritability based on the among maternal family variance component. As pointed out above, both maternal effects and deviations from the half-sib structure of the maternal families may have contributed to this discrepancy by inflating the estimated heritability. The results from a breeding design using plants from a naturalized California population of *B. rapa* indicate that maternal effects may influence estimates of the heritability of trichome production. In that population, the heritability of trichome number on the leaf edge was 0.56 based on the among-sire component of variance and 0.70 based on the among-dam component of variance (the breeding design included 60 sires and five dams/sire; Ågren and Schemske unpublished). Consequently, the realized heritability is likely to be closer to the true heritability than the estimate based on the among family variance component.

Selection on trichome number on the edge of the first leaf was effective in changing the overall trichome production of the plants, suggesting that trichome numbers in different regions of the plant are genetically intercorrelated. The maternal family means indicated a strong genetic correlation between trichome number on the edge and on the petiole of the first leaf. This correlation was reflected in the results of the selection experiment, where changes in trichome number on the edge were positively correlated with changes in the number of trichomes on the petiole. Furthermore, after seven generations of selection, the high, low and control lines had diverged in trichome production in all additional regions of the first and third leaves that were examined.

There was a positive genetic correlation between trichome production on the edge and number of days to first flower. This was shown both by the maternal family data and by the selection experiment, where high line plants began flowering 1.5 days later than control line plants at the end of the experiment. Trichome number was not significantly correlated with total flower production, suggesting that there was no trade-off between trichome number and fecundity. The rapid-cylcing population has been subject to selection for both early flowering and high female fertility for several generations. Since consistent directional selection on two traits are expected to leave antagonistically, but not positively, pleiotropic genes segregating (Falconer 1989), this has probably contributed to the negative genetic correlation between earliness of flowering and total flower number (i.e., the positive correlation between days to first flower and total flower production). Furthermore, the present genetic correlation between trichome number and days to first flower suggests that a reduction in trichome number accompanied the decrease in time to first flower during the establishment of the rapid-cycling population.

After seven generations of selection, all selected lines began flowering later than plants belonging to the base population. This may be due to inbreeding in the selected lines. The selection regime resulted in small effective population sizes, and controlled crosses have shown that selfing results in a delay of flowering in *B. rapa* (Schemske and Ågren unpublished).

Trichome production is not the only trait for which the rapid-cycling population of *B. rapa* shows considerable genetic variation. Earlier studies have documented genetic variation for isozyme loci (Williams and Hill 1986), for competitive performance (Miller and Schemske 1990) and for various physiological traits (Evans 1991).

In an experimental study we have demonstrated that trichomes significantly reduce the amount of herbivory by the cabbage butterfly *Pieris rapae* (Ågren and Schemske unpublished). That result and our finding of considerable genetic variation for trichome production in *Brassica rapa* suggest that controlled breeding for increased trichome number could be of value in reducing pest pressure in this species. Crosses between high-trichome plants and agricultural varieties followed by selection for favorable genetic combinations could improve pest resistance. In contrast to plant chemical defences which may often require costly and time-consuming assays, structural defences like trichomes are easily scored and can therefore be easily incorporated into artificial selection programs.

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References

Becker WA (1984) Manual of quantitative genetics, 4th ed. Academic Enterprises, Pullman, WA

Chew FS (1988) Searching for defensive chemistry in the Cruciferae, or, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! In: Spencer KC (ed) Chemical mediation of coevolution. Academic Press, London New York, pp 81–112

Evans AS (1991) Leaf physiological aspects of nitrogen-use efficiency in *Brassica campestris* L.: quantitative genetic variation across nutrient treatments. Theor Appl Genet 81:64–70

Falconer DS (1989) Introduction to quantitative genetics. Longman, London

Feeny P (1977) Defensive ecology of the Cruciferae. Ann M Bot Gard 64:221–234

Gómez-Campo C (1980) Morphology and morpho-taxonomy of the tribe *Brassiceae*. In: Tsunoda S, Hinata K, Gómez-Campo C (eds) Brassica crops and wild allies. Jpn Sci Press, Tokyo, pp 3–32

Hill WG (1972) Estimation of realized heritabilities from selection experiments. I. Divergent selection. Biometrics 28:747–765

Koornneef M, Dellaert LWM, van der Veen JH (1982) EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. Mutat Res 93:109–123

Lamb RJ (1980) Hairs protect pods of mustard (*Brassica hirta* 'Gisilba') from flea beetle feeding damage. Can J Plant Sci 60:1439–1440

Lamb RJ (1989) Entomology of oilseed *Brassica* crops. Ann Rev Entomol 34:211–229

Levin DA (1973) The role of trichomes in plant defense. Quart Rev Biol 48:3–15

Marks MD, Feldmann KA (1989) Trichome development in *Arabidopsis thaliana*. I. T-DNA tagging of the Glabrous1 gene. Plant Cell 1:1043–1050

Miller TE, Schemske DW (1990) An experimental study of competitive performance in *Brassica rapa* (Cruciferae). Am J Bot 77:993–998

Nishi S (1980) Differentiation of *Brassica* crops in Asia and the breeding of 'HAKURAN' a newly synthesized leafy vegetable. In: Tsunoda S, Hinata K, Gómez-Campo C (eds) Brassica crops and wild allies. Jpn Sci Press, Tokyo, pp 133–150

Norris DM, Kogan M (1980) Biochemical and morphological bases of resistance. In: Maxwell EG, Jennings PR (eds) Breeding plants resistant to insects. Wiley, New York, pp 23–61

Renwick JAA (1988) Comparative mechanisms of host selection by insects attacking pine trees and Crucifers. In: Spencer KC (ed) Chemical mediation of coevolution. Academic Press, London New York, pp 303–316

Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed. WH Freeman, New York

Southwood R (1986) Plant surfaces and insects – an overview. In: Juniper B, Southwood R (eds) Insects and the plant surface. Arnold, London, pp 1–22

Stipanovic RD (1983) Function and chemistry of plant trichomes and glands in insect resistance. In: Hedin PA (ed) Plant resistance to insects. Am Chem Soc Symp Ser 208, Washington D.C., pp 69–100

Via S (1984) The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and across host plants. Evolution 38:896–905

Williams PH (1985) CrGC resource book. Department of Plant Pathology, University of Wisconsin-Madison

Williams PH, Hill CB (1986) Rapid-cycling populations of *Brassica*. Science 232:1385–1389