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Inheritance of Variegation in *Collinsia heterophylla*

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ABSTRACT

Three types of variegation of *Collinsia heterophylla* Buist. (2N = 14), all controlled by recessive nuclear genes, and all transferable to the offspring through the female and male gametes, are reported: (1) Orange variegation, controlled by the reversible vao gene producing orange and green sectored or true breeding wholly orange plants. The orange phenotype is the result of significantly fewer chloroplasts in the cells of these plants. (2) White variegation (vaw), which is characterized by yellow sectors that turn white as the leaves mature; survivalship of white and green variegated plants is greatly reduced. (3) Blending variegation (vab), with indistinct (blending) borders between yellow-green and green sectors of the leaves; the yellow-green areas tend to expand as the variegated plant matures.

INTRODUCTION

The first record of variegation in the genus *Collinsia* is found in the earliest report on the hybridization experiments with *C. bicolor* (synonym for *C. heterophylla*) and *C. tinctoria* conducted by Rasmuson (1920).

The yellow variegation of *C. tinctoria* behaved in crosses as a monogenic recessive trait transferable to offspring through the female and male gametes. Leaves of the variegated plants varied from pure yellow, through variable mixtures of yellow and green, to green with small spots and/or stripes of yellow. Rasmuson attributed the increase of green sectors in the leaves of yellow plants to the operation of a dominant gene I. The I/I and I/i variegated plants having leaves with green sectors were viable, but the homozygous recessive i/i variegated plants, being yellow or nearly so, were dying prematurely. Rasmuson did not exclude the possibility of a wild type individual appearing among the offspring of a self-pollinated variegated plant.

Orange variegation of *C. heterophylla* Buist. (Scrophulariaceae) reported below, exhibited a great degree of similarity in the phenotypic expression, as well as in the inheritance pattern, with the variegation of *C. tinctoria* described by Rasmuson.

MATERIALS AND METHODS

Seeds used in these investigations were of the same source as the seeds used in previous studies (Gorsic 1973, 1977). Greenhouse cultural practices, hybridization methods and statistical tests employed for genetic analysis of *C. heterophylla* have been recently outline by Gorsic (1994).

The chloroplast counts were made from epidermal peels using the abaxial surface of mature leaves. The leaves were taken from the 8-10th node of each plant. Guard cells were observed under a 100X objective lens. The number of chloroplasts and their relative size were observed. Ten guard cells from each of three leaves of each phenotype were analysed.

RESULTS

Phenotypes of the three variegation types of *C. heterophylla*, reported in this article, are compared in Table 1.

Orange variegation, vao. Two self-pollinated sibling plants of the culture h79301 produced a total of 44 green and 12 variegated offspring. The coloration of leaves of the 12 variegated plants varied from pure orange, through combinations of predominantly orange with some green, to predominantly green with some orange streaks.

The self-pollinated orange plants produced offspring whose leaves were pure orange or orange with some green sectors (Fig. 1A). Many of these second generation orange plants died prematurely. In progenies of the self-pollinated orange-green variegated plants a correlation was observed: plants having a higher proportion of green sectors in the leaves produced a higher number of the wild type individuals among their offspring.

The reciprocal crosses between the wild type and any orange variegated plant produced non-variegated offspring, indicating that the orange variegation was a recessive trait transferable through the female and male gametes. The F_2 and backcross progenies segregated into green and variegated (including few pure orange) plants in erratic ratios: most cultures deviated but little from the classical monohybrid ratio, whereas in some cultures the deviations were significant or highly significant (Table 2).

Plants of the recessive class of some F_2 cultures were all pure orange leaved (lack of vao reversion in all plants), or all had orange-green sectored leaves (vao reversions in all plants), whereas in F_2 cultures of other hybrids the recessive class contained both aforementioned phenotypes (vao reversions in some plants, none in others). Two cultures (h9384, h9385), a selfing and an intercross of two heterozygous (Vao/vao) sibling plants produced a total of 191 plants, of which 144 were wild type, 32 had orange-green sectored leaves, and 15 plants had pure orange leaves. The number of individuals in these phenotypic classes fit the modified F_2 ratio of 12 (143 vs. 144) : 3 (36 vs. 32) : 1 (12 vs. 15) indicative of a dihybrid with a dominant epistatic gene ($X^2 = 1.192$, $P = .5$). The vao gene reversion to the wild type allele, it appears, required an activator (Ac) gene independent of the vao gene: Vao/vao Ac/ac - 9 Vao–Ac-- (green) : 3 Vao --ac/ac (green) : 3 vao/vao Ac -- (orange-green sectored) : 1 vao/vao ac/ac (pure orange). A self-fertilized

orange variegated plant having orange-green sectored leaves (culture h94227) produced 38 plants. Of these, 31 were either green (2) or orange-green sectored (29), and 7 plants which had pure orange leaves. The likely genotype of this orange variegated plant was: vao/vao Ac/ac. Further studies of the Ac gene's role in variegations in *C. heterophylla* are under way.

Chloroplast analysis indicates that Vao controls the number of chloroplasts in the cells. Pure orange plants contain a mean of 3.9 chloroplasts per guard cell compared with 12.1 chloroplasts per guard cell in wild type plants. In variegated plants, 3.0 per guard cell were observed in the orange-yellow sectors whereas 10.2 chloroplasts per guard cell were detected in the green sectors of the leaves. In addition to the mean number of chloroplasts differing between the green and orange-yellow tissue, the overall morphology of the chloroplasts were qualitatively different. The chloroplasts in the orange-yellow tissue appeared smaller and lighter in color when compared to chloroplasts in the green sectors of the leaves. The color may reflect differences in the number of thylakoids, the number of light traps, or the number of chlorophyll molecules per chloroplast.

White variegation, vaw. In the culture h8741 a variegated plant appeared whose leaves had yellow stripes that turned white as the leaves aged (Fig. 2B). Flowers produced in the axils of variegated bracts of this plant were self-pollinated and reciprocally hybridized with an orange variegated plant (vao/vao) and a wild type (Vaw/Vaw) plant.

Seeds of the self-pollinated flowers produced the wild type and white variegated plants in about equal proportion. The hybrid seeds, however, produced only the wild type offspring, indicating that the white variegation (vaw) and orange variegation (vao) genes were both recessive and nonallelic. The F_2 and the backcross progenies of the Vaw/vaw hybrids segregated in typical monohybrid ratios, and the selfings of dihybrids Vao/vao Vaw/vaw produced plants of four phenotypes: wild type, orange variegated, white variegated, and an orange-white-green sectored type in a 9:3:3:1 ratio (Table 3).

The white variegation trait, then, was controlled by a mutated nuclear gene vaw, nonallelic to vao, and transferable through gametes to the offspring.

The reversibility of the vaw allele has not been investigated because of the very low viability of plants with the vaw/vaw genotype.

Blending variegation, vab. In the progeny of a self-pollinated plant (culture h88105) six out of 19 plants had leaves displaying yellow-green patches or stripes. These variegated plants resembled the orange variegated (vao) plants except for having indistinct (blending) borders between the yellow-green and green areas (Fig. 2C).

The reciprocal crosses of wild type (Vab/Vab) plants and blending variegation (vab/vab) mutants produced wild type offspring. The F_2 and backcross progenies segregated in statistically acceptable monohybrid ratios (Table 3). The segregation data of cultures revealing a lack of independent assortment of vab and vao genes are given in Table 4.

The phenotypic expression of the vab/vab plants varied considerably. Some of these plants could be identified only by examining their leaves in the translucent light: variable shades of green indicated a differential transmission of light due to an uneven concentration of chlorophyll (chloroplasts) in the leaves of mildly variegated plants.

In contrast to the orange variegated (vao) seedlings, whose emerging cotyledons were partly to completely orange, the cotyledons of vab plants appeared wild type and stayed green for about two weeks after emerging from the soil when the characteristic fading of green signaled the onset of yellow-green symptoms of blending variegation.

As in the orange variegation, discussed above, the yellow-green and green sectoring of leaves followed the pattern seemingly set by the color distribution on cotyledons and the first pair of leaves. The green sectors appeared to have been produced by mitotic divisions of reverted cells of the seedling's apical meristem, rather than arising from random reversions of vab genes in cells of the leaves.

DISCUSSION

In this current investigation of genetic variability and gene linkage studies in *C. heterophylla*, four different variegation types have been encountered. The basic genes involved in the chlorophyll and/or chloroplast formation for three of these variegations have been identified.

The experimental results indicate that the primary hereditary components controlling variegations were located in the nuclear chromosomes rather than in the DNA of cytoplasmic organelles. Of the three variegation types investigated, only the orange (vao) and blending (vab) type have been afforded a closer look.

Orange variegation, vao. Reciprocal crosses indicate that orange variegation is a recessive trait controlled by a nuclear gene. The F_2 and backcross progenies included green and variegated plants. Most cultures deviated little from the classical monohybrid ratio, whereas in some cultures the deviations were significant (Table 2). These results are most easily explained by assuming that the recessive vao allele is subject to gene reversion, likely by a transposable element. Presumably, pure orange plants were produced by vao/vao individuals lacking a specific transposable element or an activator for it (Table 2).

By the close observation of the distribution pattern of the orange and green areas in stems and leaves of the variegated plants, the time of the vao gene reversion may be surmised. In the vao/vao seedlings displaying large areas of green on their cotyledons, the vao reversion to the wild type Vao allele must have occurred at an early precotyledonous stage of embryonic development. Leaves of these plants were predominantly green exhibiting some patches or stripes of orange color (Fig. 1B). In plants having both the cotyledons and the lowermost leaves predominantly orange, and patterned sectors of green on the leaves higher-up the stem, the vao reversions must have occurred either (1) at the inception of cotyledon and epicotyl differentiation of the embryo, and the green tissues of cotyledons and stem (leaves) resulted from mitotic propagation of the reverted

chlorophyllous cells of the chimeric embryo by the sorting out process during the organogenesis of cotyledons and leaves, or (2) the gene reversions may have occurred after the inception of primordia in the embryonic cotyledons and leaves themselves. The localization of leaves with green sectors in one or two rows on the same side of the stem (Collinsias have decussate leaf arrangement), and the retention of approximately the same proportion of orange to green areas in the leaves and bracts along the stem, indicate that vao reversions occurred in the apical meristem, rather than in the growing leaves.

Variegated plants having leaves that exhibited green patches and small green spots scattered all over the predominantly orange blade (Fig. 2A), suggested gene reversions are taking place in the developing leaves themselves. However, the scattering pattern may be the result of transposition of transposable elements occurring during the mitotic chromosomal replications (in young embryo) affecting only one of the newly formed chromatids (Fedoroff 1984). This reversion would produce revertant and nonrevertant daughter cells that would give rise to the chimeric apices with a more or less even distribution of revertant cells. The leaf primordia of such apical chimeras would reflect the same mixture of cells and would develop into mature leaves exhibiting a scattered distribution of the patches and spots of green over the orange blade (Fig. 2A).

Appearance of the wild type individuals among the offspring of the self-pollinated variegated (vao/vao) plants can be explained by the production of revertant Vao gametes in the flowers of chimeric (Vao/vao vao/vao) peduncular apices, or by gene reversions occurring in the zygotes.

Lower than the expected number of the variegated plants counted among the F_2 progenies of some hybrids (Table 2) were attributed, in part, to the change in gene frequency in favor of the Vao allele, due to the reversion of vao genes. The absence of variegated plants from the F_2 progenies of other hybrids (e.g. sample h89416, Table 2) can be ascribed to either (1) the virtual homozygosity (Vao/Vao) of "hybrids", brought about by the donation of revertant Vao gametes by the recessive parents at the time of fertilization, or (2) to the reversion of the vao genes taking place in the heterozygous zygotes themselves or in the very young embryos.

Apparently only the recessive vao alleles were amenable to reversion. In hundreds of the Vao/vao hybrids examined not one variegated plant appeared that would indicate the reverse allelic change. The revertant Vao alleles appeared as stable as their wild type counterparts.

Blending variegation, vab. Plants with well expressed blending variegation (vab) bred true, and, when crossed with the orange variegated (vao) plants, produced the wild type offspring. The F_2 progenies of these hybrids segregated into the wild type and variegated plants of the blending and orange type in an approximate ratio of 2:1:1. It was later demonstrated by backcrosses that the absence of the double recessive plants from the F_2 progeny was due to a tight linkage of vab and vao genes in repulsion phase (Vabvao/vabVao), which resulted in a 2:1:1 ratio for the wild type and the two variegation types, blending and orange (Table 4).

Results from reciprocal crosses between homozygous wild type plants and blending variegation mutants, and between orange variegated mutants and blending variegated mutants indicate that blending variegation was controlled by a mutated nuclear gene vab, nonallelic with vao, and transferable to the offspring through the female and male gametes. The allelic relationshp between the white variegation gene vaw and blending variegation gene vab has not been investigated.

The yellow-green areas of leaves of the blending variegation plants showed a distinct tendency to expand - apparently by diffusion of a vab controlled substance - rendering entirely yellow-green plants of low fertility.

To explain these results, the bidirectional gene conversion of the whole-chromatid and half-chromatid conversion type (à la *Neurospora crassa*), demonstrable in heterozygotes, was excluded from the consideration as being a possible mechanism of the vao and vab restoration to the wild type alleles, because in the orange and blending variegation mutants no bidirectional change has been observed in heterozygotes.

The gene reversion by a transposable element agreed best with the behavior of the vao and vab alleles in their mutability as reflected in the coloration patterns observed in stems and leaves of the variegated plants.

Plants with orange and green (vao) and yellow-green and green (vab) sectored leaves represented those homozygous recessive (vao/vao and vab/vab) individuals in which somatic vao and vab reversions occurred either during embryogenesis or after the active growth has been resumed at germination of seed - or both. Random gene reversions, occurring during the postgermination period would render a random distribution of color sectors on the leaves. In *C. heterophylla* this has not been observed. Observations supporting the pregermination reversions in variegated plants are: (1) coloration pattern of cotyledons and the first pair of leaves (presence vs. absence and the proportion of colored sectors) seem to set the coloration pattern for the remaining vegetative leaves and bracts, (2) the restriction of a specific color to leaves of one or two rows on one side of stem, and (3) the alternate leaf pairs (positioned one upon the other in the same plane) mimicking one another in amount and/or localization of sectors (left vs. right side of leaf blade). Factors favoring gene reversion during embryogenesis are not known. The influence of maternal genotype may be one of them.

The orange variegation of *C. heterophylla* is an unstable mutant similar to flavostriata of *Antirrhinum majus* investigated by Delool et al. (1986) except that in *C. heterophylla* a stable recessive, true breeding, orange mutant has been isolated. The reversibility of vao gene appears to follow the controlling (transposable) element model of a defective structural gene component (vao) as the receptor and an autonomous regulator component (Ac) (Nevers *et al*. 1986). Rasmuson's (1920) I gene of *C. tinctoria* may also have been such a regulator.

The yellow-green areas of leaves of the blending variegation (vab) mutants showed a clear tendency to expand (on account of green sectors) during the vegetative growth, which makes the vab gene suspect of being involved in chlorophyll (chloroplast) destruction in addition to being a defective gene involved in its synthesis. If so, then the yellow-green patches of leaves may have been simply the areas of vab-guided synthesis of a diffusable substance capable of speeding up the breaking down of chlorophyll (chloroplasts) or slowing down its synthesis. In young mildly variegated plants (with high vab reversion rate) having less than 1/4 of leaf area yellow-green, the symptoms did not seem to expand to the deleterious level, and plants matured properly. In young plants (with low vab reversion rate) with more than 1/2 of the surface area of leaves yellow-green the leaf discoloration was enhanced, so that at the flowering time the upper vegetative leaves and bracts of the peduncle were devoid of nearly all green color. Such plants produced mostly nonviable seed. At this stage of investigation it is not clear whether the diffusible substance acted directly on the anabolic or catabolic processes of chlorophyll metabolism or indirectly via nuclear and/or cytoplasmic DNA.

No stable, true breeding, wholly yellow-green (vab) plants have as yet been isolated.

Orange (vao) and blending (vab) variegation mutants were crossed with yellow, another recessive leaf pigmentation mutant of *C. heterophylla* controlled by the nonreversible gene y (Gorsic 1994). Fertile yellow plants (yy) were produced and the coloration pattern of the double homozygous recessives, vao/vao y/y and vab/vab y/y, were examined. Seedlings of these double recessives exhibiting no vao or vab gene reversions had bright yellow cotyledons which turned gradually pale yellow, became albino-like and died within 10 days after emerging from the soil. The double recessives having reverted vao^R or vab^R alleles respectively produced greenish-yellow and bright-yellow sectored cotyledons and leaves having sharp borders between the color sectors in vao^R/vao y/y individuals and with blending borders in vab^R/vab y/y plants (bright-yellow sectors turning pale-yellow and white with age). A triple homozygous recessive vao^R/vao vab^R/vab y/y produced intensely greenish-yellow and bright-yellow sectored cotyledons and leaves with sharp borders between some color sectors and blending between others.

In all these double and triple homozygotes, the lower the rate of vao and/or vab gene reversion, the larger were the bright-yellow (pale-yellow and white) areas of cotyledons and leaves and the lower was the survivalship; and vice versa, the greater the rate of reversion, the larger were the greenish-yellow areas of cotyledons and leaves and the higher was the survivalship of plants.

From these observations it can be concluded that the double and triple recessive plants regarding the y, vao and vab genes (vao/vao y/y, vab/vab y/y, vao/vao vab/vab y/y) without reversion of either of the variegation genes cannot produce chlorophyll (chlorplasts) and die after they exhaust the food supply of their cotyledons. That means that the y gene is controlling a certain step in chlorophyll synthesis (chloroplast formation) which requires the products of the dominant Vao and Vab alleles that their recessive counterparts are unable to provide. The same applies - mutatis mutandis - to the

vao and vab alleles in their relation to the y gene. The decreased number of chloroplasts in the guard cells and their altered morphology supports this hypothesis.

If a transposon was involved in evoking the original vao allele (of this investigation), which behaves as a stable gene in some genotypes, then the question may be raised: Is the transposition of transposable elements one of the routine processes of generating new alleles in natural populations?

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Table 1. Types of variegation in *Collinsia heterophylla*

Name and Genotype	Phenotype
1. Orange, vao/vao	Stems and leaves either (1) uniformly orange, (2) predominantly orange with some green patches or stripes, (3) predominantly green with some orange-yellow patches or stripes, or (4) orange-yellow with scattered green patches and spots. The last three patterns are expressed in individuals with vao gene reversions.
2. White, vaw/vaw	Stems and leaves of young plants green with yellow patches and/or stripes turning white with age. The vigor of these plants is greatly reduced.
3. Blending, vab/vab	Stems and leaves exhibiting yellow-green pathces or stripes having blending borders with green; yellow-green sectors tend to expand, occasionally rendering entirely yellow-green leaves. The growth rate of the predominantly yellow-green plant is reduced.

Table 2. Phenotypic segregation of F_2 and backcross (BC) progenies of orange variegated hybrids of *Collinsia heterophylla* (Ratios: F_2 3:1, BC 1:1, * Seed parent vao/vao)

Culture	Progeny	# green	# variegated	Total	Chi-square	P
86181	F_2	100	29	129	0.436	.5
8851	F_2	89	22	111	1.588	.2
8882	F_2	61	9	70	5.505	.02
8884	F_2	26	9	35	0.007	.95
89395	F_2	24	2	26	3.283	.1
89397	F_2	45	10	55	1.364	.2
89398	F_2	66	6	72	10.667	.001
89399	F_2	35	8	43	0.939	.3
89456	F_2	28	7	35	0.549	.4
90111	F_2	72	17	89	1.650	.2
9219	F_2	29	4	33	2.272	.1
9220	F_2	50	6	56	5.356	.02
9221	F_2	39	11	50	0.106	.7
9222	F_2	25	9	34	0.000	---
9238	F_2	27	5	32	1.041	.3
9247	F_2	148	47	195	0.083	.8
9349	F_2	18	3	21	0.777	.3
9378	F_2	27	8	35	0.009	.95
9380	F_2	51	13	64	0.520	.4
93108	F_2	24	9	33	0.009	.95
93109	F_2	41	10	51	0.529	.5
94109	F_2	20	4	24	0.500	.5
94126	F_2	17	3	20	0.600	.4
94246	F_2	37	6	43	2.797	.1
8107*	F_2	174	48	222	1.352	.2
8673*	F_2	98	26	124	1.074	.3
89416*	F_2	47	0	47	14.235	<.001
89418*	F_2	53	3	56	11.524	<.001
89423*	F_2	14	4	18	0.000	---
93176*	F_2	116	31	147	1.198	.25
8814	BC	6	5	11	0.000	---
89386	BC	7	9	16	0.062	.7
89387	BC	7	9	16	0.062	.7
89461	BC	11	8	19	0.210	.5
9426	BC	10	13	23	0.172	.2
9429	BC	11	13	24	0.040	.7
94147	BC	12	13	25	0.000	---
94148	BC	13	16	29	0.137	.7
83107-8	BC*	7	3	10	0.900	.25
8403	BC*	5	5	10	0.000	---
89404	BC*	3	3	6	0.000	---
9432	BC*	4	4	8	0.000	---

Table 3. Phenotypic segregation of monohybrids and dihybrids of white (vaw) and blending (vab) variegation mutants of *Collinsia heterophylla*.

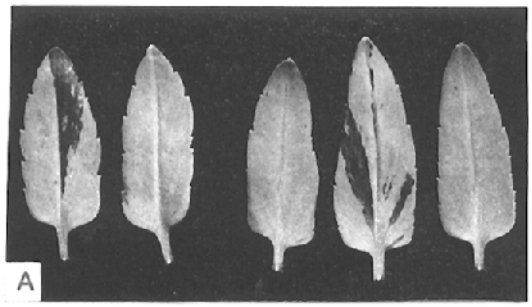
Culture	Genotype/Cross	Progeny[a]	Green	Varie-gated	Varie-gated	Double Reces.	Total	Chi-Square	P
8844	Vaw/vaw	F_2	9	1	–	–	10	0.533	0.5
89546	Vaw/vaw	F_2	7	3	–	–	10	0.000	---
89548	Vaw/vaw	F_2	9	2	–	–	11	0.029	0.7
8850	Vaw/vawxvaw/vaw	BC	6	10	–	–	16	0.562	0.4
89544-48	Vao/vao Vaw/vaw	F_2[b]	29	10	9	1	49	0.839	0.8
86114	Vab/vab	F_2	9	–	1	–	10	0.533	0.4
88105	Vab/vab	F_2	13	–	6	–	19	0.680	0.4
9223	Vab/vab	F_2	21	–	7	–	28	0.000	---
9240	Vab/vab	F_2	36	–	4	–	40	4.033	0.02
9247	Vab/vab	F_2	47	–	1	–	48	12.249	<0.001
94122	Vab/vab	F_2	24	–	6	–	30	0.177	0.7
94362	Vab/vab	F_2	12	–	3	–	15	0.016	0.9
94365	Vab/vab	F_2	11	–	4	–	15	0.129	0.7
93254*	Vab/vab	F_2	6	–	2	–	8	0.000	---
9459*	Vab/vab	F_2	9	–	5	–	14	0.380	0.5
94367*	Vab/vab	F_2	6	–	3	–	9	0.286	0.6
94368*	Vab/vab	F_2	8	–	2	–	10	0.000	---
9368	Vab/vabxvab/vab	BC	4	–	4	–	8	0.000	---
94301	Vab/vabxvab/vab	BC	4	–	4	–	8	0.000	---
94396	Vab/vabxvab/vab	BC	30	–	23	–	53	0.924	0.35
94397	Vab/vabxvab/vab	BC	4	–	3	–	7	0.000	---
94398	Vab/vabxvab/vab	BC	8	–	7	–	15	0.000	---

[a] Expected ratios: F_2 3:1; BC 1:1; [b] Dihybrid F_2 9:3:3:1 * Maternal parent vab/vab

Table 4. Phenotypic segregation of F_2 progenies of dihybrids (Vao/vao Vab/vab) of orange (vao) and blending (vab) variegation mutants of *Collinsia heterophylla* indicating vao-vab linkage[a].

Culture	Green	Orange varieg.	Blending varieg.	Double varieg.	Total	Chi-square	P
89440-5	21	8	6	---	35	1.099	0.5
9441	9	3	3	---	15	0.165	0.5
9460	23	9	9	---	41	0.825	0.7
9365	8	2	5	---	15	0.565	0.7

[a] The absence of double recessives due to vao-vab linkage in repulsion phase (Vaovab/vaoVab) rendering a 2:1:1 ratio instead of the expected 9:3:3:1 ratio.



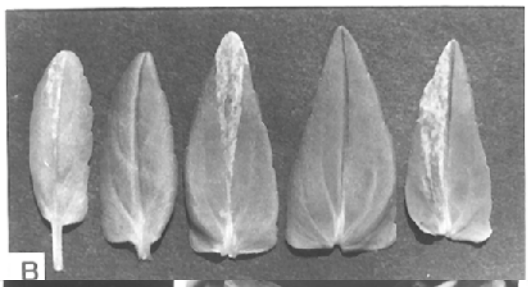


Figure 1.
Orange variegation (vao) in *Collinsia heterophylla.*
A. Pure orange (no vao rever-sion) and orange green sec-tored leaves (with vao rever-sion) - two (left) from lower, three (right) from upper node. B. Leaves from 3-4-5-6-7th node from same side of stem (notice orange sectors on alternate leaves).

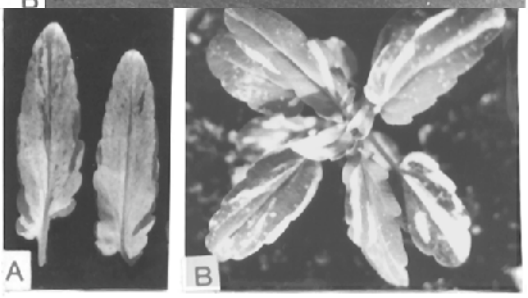


Figure 2.
Three variegation types of *Collinsia heterophylla.*
A. Orange variegated (vao) leaves with patches and spots of green in orange blades.
B. a white green variegated (vaw) mutant.
C. Blending variegation (vab). Leaf pairs (left to right) of the 1-2-3-4th node exhibiting reduction of green areas and expanding of yellow-green areas.