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Inheritance and Reversion of Floral Transformation in *Collinsia heterophylla*

J. Goršič
Elmhurst College
Department of Biology
Elmhurst, IL 60126-3296

ABSRACT

The pleiotropic transformer (*trf*) gene of *Collinsia heterophylla* Buist., 2N=14, (Scrophulariaceae), modifies all floral parts rendering the flower sterile. A dominant activator (*Act*) gene, linked with *trf*, and an active transposable element (Tel^a) are required for the expression of maroon pigmentation, as well as sepaloid texture of leaves, and green leaf formation in orange (*vao*) plants – all associated with the transformed trait.

INTRODUCTION

During the vegetative growth, *Collinsia heterophylla*, Buist. (Scrophulariaceae) plants develop five to eighteen pairs of leaves. The basal and medial areas of cotyledons and juvenile leaves of most of the red-stemmed plants exhibit transient maroon patches (Fig.1). The transition to the reproductive phase is displayed by an increase in the number of leaves from two to three, four, or five at the lowermost whorl of inflorescence. The rest of verticillate inflorescence is composed of trimerous, tetramerous to as high as heptamerous whorls of bracts each subtended by a flower (Goršič, 1974). The wild-type pentamerous, zygomorphic flowers (Fig.2) have a white, two-lobed upper lip (with maroon dotting) and a violet lower lip having two side lobes and a longitudinally folded middle lobe (keel) harboring four didynamous stamens and a two-carpellate pistil (Abrams and Ferris, 1960).

MATERIALS AND METHODS

Among the progeny of 144 plants of six self-fertilized and intercrossed sibling plants (culture h90111), grown in the greenhouse for the purpose of ongoing study of genetic variation in the species, eight mutant plants appeared having the following unusual features: 1) leaves of the fourth and higher stem nodes had a maroon colored adaxial side of the blade (Fig.3); 2) the fourth, fifth, and sixth node had four (instead of the usual two) branches carrying five to six pairs of tightly packed sepaloid leaves, or having five to six pairs of sepaloid leaves and terminal “incomplete flowers” composed of five sepaloid and five petaloid bracts (two or more of the latter with maroon dots), four staminoid bracts, and no pistil; 3) the branches (two per node) at the seventh and eighth node had four or five pairs of sepaloid leaves and a terminal “incomplete flower”; and 4) the sepaloid leaves of the ninth and the higher nodes of the main stem were compressed into a “cone-

like" structure with "incomplete flowers" (Fig. 4). These transformed (*trf*) plants appeared stunted, reaching about one-third size (15cm) of the wild-type sibling plants, and were, except one, sterile. The fertile transformed (*trf*) mutant had, in addition to most features described above, on the branches of the sixth node normal flowers and fully developed, but transformed, sterile flowers in the upper part of the flowering stalk (Fig.5). In subsequent cultures of self-fertilized transformed (*trf*) mutants the majority of transformed plants developed wild-type revertant ($trf^{+} = trf^{r}$) flowers on the lower and the transformed (*trf*) flowers on the upper branches (Fig.6). These transformed flowers drastically differed from the wild-type flowers by having a soft, papery calyx, more intensely colored (often patchy) corolla with a half-open keel, four short sterile stamens, and a short sterile pistil.

To establish the genetic basis and the mode of inheritance of transformed (*trf*) trait, the fertile mutants were self-fertilized and hybridized with the wild-type (*Trf*) plants. The results of these investigations are reported below. The cultivation of *Collinsia* plants and hybridization methods have been described in an earlier paper (Goršič, 1994).

RESULTS

Plant Maturation and Transformation Levels in Transformed Mutants

The transformed (*trf*) mutant (h9247-1), from which the bulk of the transformed offspring of this investigation are derived, had the following mature configuration: one of the two branches at the sixth stem node bore at its third and all higher nodes only normal flowers, the other had at its third node normal flowers and transformed flowers at all remaining nodes; two branches of the seventh stem node carried at their third and all higher nodes only transformed flowers; at the eighth and all higher nodes of the main stem there were only transformed flowers. The reproductive maturity (flower formation) in this plant, having seven pairs of vegetative leaves, has been reached at the eighth node on the main stem, at the ninth node (6+3) on branches of the sixth stem node, and the tenth node (7+3) on the branches of the seventh node. The change from normal to transformed flowers occurred on the main stem apparently at the eighth node, but at the tenth node (6+4, 7+3) on the branches (for more on maturation see below).

Crosses between wild-type (*Trf/Trf*) plants and the transformed (*trf/trf*, h9247-1) pollen plant produced wild-type F_1 offspring. Among the progeny of 70 offspring, of the self-fertilized hybrids (*Trf/trf*), not one transformed (*trf*) plant reappeared. A self-fertilized wild-type (*Trf/trf*) sibling plant of the h9247-1 transformed (*trf*) mutant produced 162 wild-type and two transformed plants (culture h9384). Five wild-type siblings of culture h9247 crossed with the h9247-1 transformed mutant produced a total of 92 plants of which 88 were wild-type and four were transformed plants (cultures h9385-89).

On the basis of these preliminary observations it can be concluded that the transformed (*trf*) is a recessive trait, and it is not inherited as a monogenic, nor a simple bi-, or tri-genic trait, but conditioned by an intricate interaction of three or more genetic factors or by an epigenetic mechanism interfering with normal development of flowers in *C. heterophylla*.

Seeds (labeled *trf*^{6}) obtained from self-fertilized flowers of the branch at the sixth stem node of the h9247-1 transformed (*trf*) plant, when sown, gave rise to ten wild-type green-leaved plants and four maroon-leaved transformed (*trf*) plants (cultures h9382,3). The wild-type siblings began to form flowers on the main stem at the ninth to twelfth node, and the transformed (*trf*) mutants at the eleventh to twelfth node, except one plant whose main stem was terminated by a "cone" of sepaloid bracts. The flower formation on the primary and secondary branches of transformed (*trf*) plants started at a higher total number of nodes than on the stem (confer Fig.7): on the primary branches of the ninth stem node at the 14th or 15th node (9+5, 9+6), on branches of the 10th stem node again at the 14th or 15th node (10+4, 10+5), and on branches of the eleventh stem node at the twelfth node (11+1). On the secondary and tertiary branches the total number of nodes at which the flowers appeared add up to the values 14 to 18. In *C. heterophylla* plants, apparently, the main stem matures at the same or a lower node number (12) than the branches. The pleiotropic transformer (*trf*) gene, then, does not interfere with the plant maturation process, but with the physiological (pigment synthesis) and morphological (leaf texture) transformation of vegetative organs (leaves) to the reproductive structures (bracts and floral parts).

Discoloration of Leaves in Transformed Mutants

The earliest sign that a plant is a transformed (*trf*) mutant is the appearance of a conspicuous maroon pigmentation of leaves, which may occur as early as the second node (Fig.8), middle (4th and 6th node, Fig.3), or in late vegetative phase (11th node, Fig.9), or when the plant is already in the reproductive phase (Fig.10). The pigment is formed only in the cells of the upper epidermis; the concentration varies from cell to cell and some cells are colorless. After the onset of pigmentation, the intensity as well as the number of pigmented cells per unit area increase for several newly formed leaf pairs. As the reproductive maturity approaches, the leaves are gradually reduced to bracts of inflorescence, and the maroon color fades. With inbreeding of transformed (*trf*) plants the intensity of maroon pigmentation increased, supporting the view that maroon coloration may be a polygenic or multiple allelic trait. Those red-stemmed violet (*W*) and green- or red-tinge-stemmed white (*w*) flowering (Hiorth, 1930) transformed mutants that exhibited no maroon pigmentation apparently lacked some genetic factor necessary for synthesis of the pigment; these transformed (*trf*) plants were nevertheless recognizable in the young as well as mature stage by the modified, sepal-like texture of leaves (Fig.4).

Segregation in Progenies of Selfed and Intercrossed Transformed Mutants

The configuration of the offspring of self-fertilized and intercrossed transformed (*trf)* plants were basically of four types: 1) plants having on the main stem normal flowers in the lowermost whorls of inflorescence and transformed flowers in the upper part (occasionally a plant had a normal flower in the middle of transformed inflorescence); 2) transformed plants having on the main stem and the uppermost branches only transformed flowers and normal flowers on the lower branches; 3) plants having no fertile flowers because a) they produced only transformed flowers on the main stem and no branches, b) produced on the main stem and all branches only transformed flowers and c) produced only "incomplete flowers" in "cones" on the main stem and branches; 4) plants with green leaves and wild-type fertile flowers only.

A correlation has been observed between the level (node) of appearance of the first maroon leaf pair and the node number at which the branches with transformed flowers developed. Branches that carried only transformed flowers developed (generally) at one to three nodes beneath the lowermost node with maroon leaves, e.g., a plant having the lowermost maroon leaves at the fifth stem node had transformed flowers on the branches of the third and all higher nodes – such a plant had normal flowers on the branches (if any) at the second, first, and/or the cotyledonous node (seed collected from such a plant was labeled *trf*^{2} and seed of all fertile branches were clumped together).

The segregation data of selfings and intercrosses of transformed (*trf*) plants (Table 1) displayed a correlation: progenies grown from seeds collected on the branches at higher stem nodes produced larger number of wild-type green (revertant, *trf*^{r}) plants than progenies derived from seeds collected on branches at the lower stem nodes, and vice versa, the lower on the stem the seed was collected the higher was the number of transformed (*trf*) maroon-leaved plants, and the lower the number of green-leaved (revertant, *trf*^{r}) plants in the progeny.

The transformed (*trf*) classes (of non-segregating as well as *trf*^{r} vs. *trf* segregating cultures) were always a mixture of individuals regarding the level (node number) at which the shift from wild type (*trf*^{r}) to transformed (*trf*) flowers appeared. Selfings and intercrosses of plants with transformed flowers on lower branches (1st-5th node) produced a higher number of offspring having the transformed flowers on lower branches than selfings and intercrosses of plants carrying transformed flowers on branches at the 6th-10th (or higher) node, whose progenies, generally, contained a greater number of plants with transformed flowers in the upper branches. Transformed classes, whose members would all exhibit the shift from normal to transformed flowers on branches of the same stem node, have not been observed.

Segregation in Progenies of Selfed and Intercrossed Revertant Plants

Progenies of the self-fertilized and intercrossed wild-type green (revertant, *trf*^{r}) plants were of five segregation types (Table 2): 1) progenies composed entirely of revertant (*trf*^{r}) plants; 2) progenies segregating for green-leaved (*trf*^{r}) and maroon-leaved transformed (*trf*) plants in exceedingly skewed ratios favoring revertant (*trf*^{r}) type; 3) progenies not significantly deviating from a 3:1 ratio for revertant (*trf*^{r}) vs. transformed *(trf*) type; 4) progenies approaching a 1:1 ratio for revertant (*trf*^{r}) vs. transformed (*trf*) type; and 5) progenies in which the transformed (*trf*) plants outnumbered the revertant (*trf*^{r}) type. Members of the transformed (*trf*) classes varied as in cultures of selfings of transformed plants. The revertants (*trf*^{r}) were phenotypically identical with *Trf* wild-type plants, but the *trf*^{r} alleles did not behave as dominant *Trf* genes in selfings and crosses with transformed (*trf*) plants; therefore the *trf*^{r} genes cannot be considered stable *Trf* genes.

Segregation in Progenies of Crosses Between Revertant and Transformed Plants

In crosses between revertant (*trf*^{r}) and transformed (*trf*) mutants the revertant plants were used predominantly as maternal parents for a simple reason of having many more fertile flowers (assuring higher yield) than the transformed plants, which often had a very limited number of normal flowers, and were therefore used primarily as pollen parents (Table 3). In spite of enormous variation in genomic make up of revertant (*trf*^{r}) plants, as demonstrated by great fluctuations in segregation ratios of their selfings (confer Table

2), the segregation data of progenies of *trf*^{r} x *trf* and *trf* x *trf*^{r} crosses did indicate a regularity in obtaining higher proportion of revertants (*trf*^{r}) in crosses whose transformed (*trf*) parent (female or male) bore the transformed flowers at a higher level (node) on the stem, and the reverse, the lower on the stem was the position of the branch with transformed flowers in the transformed parent (female or male), the higher was the proportion of transformed (*trf*) offspring in the progeny of the cross. The "*trf*^{N} phenotypes" of parents of individuals crossed gave some information on their genetic background, but it was incomplete, since their siblings often could not be genetically tested because of their sterility.

Reversion of Orange Leaf Variegation in Transformed Mutants

The orange variegation (*vao*) is a mutiple-allelic locus comprising seven alleles (Goršič and Kerby, 1996; Goršič, 2000); two of the recessive alleles were used in this investigation – *vao* producing pure orange leaves (Figs.11,12) and revertant *vao*^{R} (whose reversion occurs during embryogenesis) producing orange and green sectored leaves (Figs.13,14,15). In cultures segregating for transformed (*trf*) and orange leaf variegation (*vao*) mutants all transformed plants, which were pure orange (*vao*/*vao*) at emergence, began to exhibit green sectored or entirely green leaves at their 2nd, 3rd, 4th, or at any other of the remaining nodes (Fig.11). In some orange transformed double mutants (*vao*/*vao trf*/*trf*), the pair of leaves in which the *vao* reversion to *vao*^{t} started was light green, but leaves of the next pair up the stem were dark green (Fig.12). The revertant orange variegated plants (*vao*^{R}), characterized by orange and green sectored leaves (Fig.13, green may be large sectors – or tiny specks, Fig.14) having the transformed (*trf*/*trf*) genotype, exhibited a drastic increase in the area of green on leaves starting at the 2nd, 3rd, 4th, or any higher stem node (Fig.14), or exhibited entirely green leaves from a specific node upwards (Fig.15). The increase in the amount of green in the leaves of *trf*/*trf vao*^{R}/*vao*^{R} double mutants may not have been a reflection of an upgraded enhancement of the *vao*^{R} allelic action, but a result of the operation of a different form of a revertant *vao*^{tR} allele in whose origin the *trf* gene was a participant.

In combined progenies of self-fertilized and intercrossed *Vao*/*vao* and *Vao*/*vao*^{R} heterozygotes of transformed (*trf*/*trf*) stock, 651 plants were obtained from which 412 were (*vao*$^{+}$-*trf*-*Act*) maroon green and transformed, 90 were (*vao*$^{+}$-*trf*^{r}-*act*) pure green with normal flowers, 47 plants were (*vao*^{t}-*trf*-*Act* or *vao*^{tR}-*trf*-*Act*) maroon pure orange – or maroon orange green sectored at the base, and maroon extended green sectored to all green – or maroon all green above with transformed flowers, and 102 were (*vao*-*trf*^{r}-*act* or *vao*^{R}-*trf*^{r}-*act*) pure orange – or orange green variegated with normal flowers. If the *vao* and *trf* genes were independent, the expected number of individuals in the four classes would be 9 (366.19) : 3 (122.06) : 3 (122.06) : 1 (40.69) respectively. Deviations from these expected numbers indicate a trans-arrangement (repulsion) of *vao*-*trf* and a dominant factor (*Act*) participating in activation of *vao* and *trf* genes (*Vao trf act* / *vao trf Act*, *Vao trf act* / *vao*^{R} *trf Act*).

Formation of Actinomorphic Transformed Flowers

One of the transformed (trf) mutants had unusual pentamerous actinomorphic flowers (Fig. 16). The immediate ancestors of this plant (h93207-13) carried the superflower gene (*sf*; Goršič, 1994), which modifies terminally located flowers into actinomorphic flowers composed of keels only; the same type of keel flower in lateral position is

produced by white fleck (*Wf*) mutant (Goršič, 1957) after an inbreeding for at least five generations (Fig. 17). Keels of the keel flowers, as well as keels of the wild-type zygomorphic flowers, are changed into the side lobes by the keelless gene (kl^p; Goršič, 1994), which opens the keel and enables the "3-side-lobed" lower lip to exhibit the phenotype of genes (like carmine, *Kn*; Hiorth, 1930) normally affecting only the upper lip, to be expressed in the lower lip as well (such flower is pictured in Fig. 6; trf^r-kl^p-*Kn*). Thus, the plant with the unusual nearly white (non-carmine, *kn*) actinomorphic flowers (Fig. 16) had this genotype: *trf*/*trf sf*/*sf* kl^p/kl^p *kn*/*kn Act*/-. Similar keel flowers were observed in *C. verna* Nutt., blue-eyed Mary (Fig. 19), a native Midwestern species, whose leaves and flowers – like in *C. heterophylla* plants – may or may not exhibit maroon pigmentation (Fig. 18,19).

DISCUSSION

The extremely variable trait of maroon patches on cotyledons and leaves (Fig. 1) is controlled by *Mp* genes which, in the wild-type plants, become silenced at the end of the juvenile phase. The maroon pigmentation shows up in the reproductively mature plants as greatly variable maroon markings on the petals of the flowers' upper lip (Figs. 2,16; Goršič, 1994): dotted mouth (*Md*), cross line (*Uc*), dotted (*Ud*), and maroon ring (*Ur*). Transformer (*trf*) gene, apparently, breaks the silencing of the *Mp* genes, and vegetative leaves continue to exhibit maroon pigmentation all the way up to the modified petaloid bracts of "incomplete flowers." The recessive orange variegation (*vao*) gene, in contrast to dominant *Mp* genes, is reverted to vao^t (in presence of the *trf* gene) producing normal chloroplasts rendering the leaves green. Appearance of the fertile wild-type plants among the offspring of self-fertilized transformed mutants, demonstrates that the *trf* gene itself is reversible (trf^r).

The unusual behavior of *Mp*, *vao*, and *trf* genes, and the irregular segregation pattern in progenies of the self-fertilized and inter-crossed transformed (*trf*) mutants (Tables 1,2,3) can be adequately explained assuming the operation of two genetic factors facilitating the reactivation of these genes: 1) a dominant activator (*Act*) which is linked with *vao-trf* group, and 2) a transposable element (*Tel*) which is independent of the group (Kunze, et al., 1997). However, if the *Act* and a stable transposable element *Tel* would be the sole autonomous agents, facilitating the *vao* and *trf* reactivation process, stable and predictable segregation ratios should be obtained in genetic testing. Instead, the proportion of maroon-leaved transformed (*trf*) plants versus green-leaved revertant (trf^r) plants in test progenies appears to be influenced by the nodal (stem) position of the branch from which the seed of the tested plants has been collected. It seems, that the transposable (*Tel*) element (playing a role in *vao* and *trf* gene reversion) behaves differently when inherited (transmitted) from the lower (juvenile), middle, or upper (mature) part of the plant, suggesting, that the transposable element changes as the plant grows.

In corn (*Zea mays*; Phoetig, 1990) the *Spm* transposable element exists in three states: the active heritable state, the inactive heritable state, and labile programmable state. The programmable elements, that can shift to the stable active or stable inactive state, are progressively imethylated as the plant grows – their transcription level is influenced by the methylation status (low when highly methylated, and high when methylation is

decreased) and their position in the shoot. In the presence of the active *Spm*^{a} (whose cytosine in DNA of the transcription start site at the 5' end is methylated) the mutator (*Mu*) gene gets methylated and the *hcf106* (high-chlorophyll-fluorescence gene, producing pale-green leaves) located in the neighborhood of *Mu* regains the capacity to produce normal chlorophyll, which results in the leaves exhibiting dark green sectors or becoming entirely green. This visible change in corn leaf coloration is a reflection of the DNA methylation status of the *Mu - hcf106* segment.

In *C. heterophylla* several genes behave as the *hcf106* of corn. In transformed (*trf*) mutants the silencing of *Mp* genes is broken and the leaves, beyond juvenile phase, exhibit maroon coloration; in orange (*vao*, *vao*^{R}) plants of *trf/trf* stock, the normal chloroplasts form and green sectored or entirely green leaves are produced. The transposable element (*Tel*) of *C. heterophylla*, involved in the reactivation of these genes, exists in at least two states: the active (*Tel*^{a}) and the nonfunctional (*Tel*^{n}) form. Since to date, in progenies of the self-fertilized transformed (*trf*) plants, neither stable heritable maroon-leaved (*trf/trf*) nor stable heritable green-leaved (*trf*^{r}*/trf*^{r}) strains have materialized, it is likely, that in the meristematic (sporogenous) tissue of the apex, the transposable element stays in the nonfunctional (*Tel*^{n}) state. In mitotically dividing and differentiating cells of the soma, however, the *Tel*^{n} converts in variable frequencies (depending on the developmental level of specific section of the growing plant) to the active (*Tel*^{a}) form.

Coding genes are, generally, silenced when their DNA gains the methyl groups, whereas the transposable elements are activated by demethylation (Martienssen et al., 2001). When DNA of the activator (*Act*) gene gets methylated, involving the active transposable element (*Tel*^{a}), the *vao* and *trf* genes, located in *Act*'s neighborhood, are affected: *vao* reverts to *vao*^{t} and normal chloroplasts are produced; the *trf* initiates the ontogenetic processes of vegetative to reproductive transformation, the silencing of *Mp* genes stops, the synthesis of maroon pigment resumes, leaves acquire a sepaloid texture, and the plant becomes a transformed (*trf*) mutant with maroon colored leaves (or green leaves in orange plants) and sterile transformed flowers.

The mechanisms, turning these reactivations on and off, include the regulatory genes which control the developmental phase change (Phoetig, 1990), the methylation and demethylation of DNA (Martienssen et al., 2001), or the epigenetic pathways, such as silencing by microRNA and interference RNAs (RNAi; Hannon, 2003).

The first pair of leaves, exhibiting maroon pigmentation, may be only half-way colored (Figs. 8,9): this is because the terminal part of the leaves matures earlier (Phoetig, 1990) - the silencing of the *Mp* genes is still in progress, therefore the terminal part is green; the basal part matures later - the silencing of *Mp* genes is terminated, the synthesis of maroon pigment resumes, therefore the basal part is maroon colored. The starting point of transformation was indicated also in some plants having four (instead of usual two) branches per node: the outer two branches (formed earlier) carried non-modified normal fertile flowers, the inner two branches (formed later) bore transformed (*trf*) flowers.

In transformed (*trf*) plants, the conversion of the nonfunctional transposable element (*Tel*^{n}) to the active *Tel*^{a} form and the accompanying methylation of DNA of the *Act* and

adjacent *vao-trf* loci may occur at any level between the 2nd and the uppermost node of the stem (Figs. 3,8-12,14-15) as demonstrated by the appearance of maroon pigmented leaves at the respective nodes and, later, the appearance of modified transformed (*trf*) flowers when plants reach reproductive maturity. Progenies of self-fertilized transformed (trf) plants segregate into maroon-leaved plants with transformed (*trf*) flowers and wild-type green-leaved revertant ($trf^{+} = trf^{r}$) plants with normal flowers in most irregular ratios (see Table 1) due to: 1) variable frequencies of Tel^{n} to Tel^{a} conversion and transcription, and 2) potency (methylation status) of genetic factors regulating developmental phase changes, which affect the transposition of transposable elements. In floral primordia, the methylated DNA of the *Act* and adjacent (vao,vao^{R})-*trf* segment is activated and the *trf* starts directing the morphological development towards sterile transformed (*trf*) flower. The wild-type green-leaved or orange-leaved (vao,vao^{R}) plants are *trf/trf Act/-* Tel^{n}/Tel^{n} individuals, that lack the proper genetic make up required for the Tel^{n} to Tel^{a} conversion and transcription, thus precluding the DNA methylation of the (vao,vao^{R})-*trf-Act* segment, therefore the floral primordia continue to develop along the wild-type path, and fertile ($trf^{+} = trf^{r}$) flowers form. If any step of the process leading to methylation of the *vao-trf-Act* DNA segment is hampered to a lesser degree in the basal part than in the middle or upper parts of the plant, then, the progenies, grown from seeds gathered from lower branches of the self-fertilized transformed (*trf-Act/trf-Act*) plants, would contain a higher number of transformed (*trf*) and a lower number of revertants (trf^{r}) than the progenies grown from seeds collected from the middle or upper parts of the plant. That is actually observed in analyzing test cultures: progenies grown from seeds of self-fertilized transformed (*trf*) plants, collected from branches at the 1st to 4th stem nodes (labled trf^{1-4}), have, generally, a high number of maroon-leaved transformed (*trf*) plants and low number of green-leaved revertant (trf^{r}) plants; seeds of the "trf^{5-8} phenotype" produce progenies in which the number of the wild-type green revertants (trf^{r}) is rising and may override the maroon-leaved transformed (*trf*) type, which actually happens, with few exceptions, in progenies grown from seeds collected from capsules of the upper ($trf^{9\text{-}over}$) branches and inflorescence of the main stem.

The reversion of orange, *vao*, and orange-green, vao^{R}, alleles in transformed (*trf/trf*) mutants in *C. heterophylla* follows a similar path as the reversion of *hcf106* gene in corn (Phoetig, 1990). The combined progenies of selfed and intercrossed *Vao-trf-act/vao-trf-Act* and *Vao-trf-act/*vao^{R}*-trf-Act* transformed (*trf*) plants segregated into four classes in a ratio indicating the *vao-trf-Act* linkage (see p.5). In progenies of individual cultures of this test, no consistent crossing-over values could be ascertained because the *vao* to vao^{t} and vao^{R} to vao^{tR} reversions require DNA methylation of the (vao,vao^{R})-*trf-Act* segment, which is facilitated by the Tel^{a} that is derived from Tel^{n} whose rate of transcription, conversion and transposition is determined by the methylation status of genes regulating the developmental phase changes (these rates change as the plant grows). In the vao^{t}*trfAct/*vao^{t}*trfAct* and vao^{tR}*trfAct/*vao^{tR}*trfAct* plants (group 47 plants, p.5) the vao^{t} and vao^{tR} reversions are observable (green colored leaves on orange plants brought about by action of vao^{t}/vao^{t} and vao^{tR}/vao^{tR} genotypes); whereas in the *Vaotrfact/*vao^{t}*trfAct* and *Vaotrfact/*vao^{tR}*trfAct* plants (412 plants, p.5) the action of the vao^{t} and vao^{tR} alleles is suppressed by the dominant *Vao* (all green) allele in all heterozygous *Vao/*vao^{t} and *Vao/*vao^{tR} plants of this group.

Transformed (*trf*) plants that produce, upon self-fertilization, only transformed offspring, are most commonly grown from seeds collected from branches at the cotyledonous and the lowermost stem nodes (trf^{1-4}). The population size of thirty-nine offspring of a self-fertilized transformed plant (the highest number recorded, culture h96308, Table 1) does not necessarily establish a pure breeding *trf*/*trf* line. Plants that bore only "incomplete flowers" in cones and plants carrying only transformed flowers even on the branches of the cotyledonous node (few have been observed) may have been genetically stable, fully transformed *trf*/*trf Act*/*Act* Tel^a/- individuals, but this could not be experimentally confirmed, because they were sterile. Most of the transformed mutants (*trf*) form some wild-type fertile flowers on lower branches. In a transformed (*trf*) plant exhibiting maroon pigmented leaves, at, e.g., the 6th stem node and up, the Tel^n to Tel^a conversion, the *Act* methylation, and the reactivation of *Mp* genes occurs at that point - this plant will produce wild-type fertile (trf^r) flowers on branches of 4th and lower nodes, and transformed (*trf*) flowers on the branches of the 5th and all higher nodes, and in the inflorescence of the main stem.

In progenies of crosses between transformed plants (*trf x trf*), the parent with lower seed collection number (trf^N) seem to exert a greater influence on determination of the transformation level (earliest occurrence of maroon leaves) of the offspring than the parent with higher seed collection number; e.g. a cross trf^2 *x* trf^6 would most likely produce a progeny similar to the offspring of a self-fertilized trf^4 plant. Segregation ratios in progenies of the selfed and intercrossed revertant (trf^r) plants were, understandably, most unpredictable – they were grown from a mixture of seeds collected from any or all branches (bottom to top) and inflorescence of the main stem. Using the same criteria (different rates of Tel^n to Tel^a conversion and transcription along growing stem, methylation status of factors controlling developmental phase changes) applied in the analyses of progenies of selfed and intercrossed transformed (*trf*) plants, one can meaningfully interpret the segregation pattern of trf^r selfings and intercrosses. Although several self-fertilized revertant (trf^r) plants produced progenies composed of revertant offspring only (maximum 33 in cultures h95131 and h98351, Table 2), no stable pure breeding trf^r/trf^r line has yet been established. The hybridization of revertant and transformed (trf^r *x trf* and *trf x* trf^r) plants produced progenies that segregated in similar manner as progenies of selfings of the revertant (trf^r) and transformed (*trf*) mutants themselves. The specific effects the methylation has on DNA of the coding genes (they are silenced when gaining methyl groups) versus the DNA of transposable elements (they are activated by demethylation) and the influence the developmental phase change has on the Tel^n to Tel^a shift and the *Act* methylation provides for a many faceted interplay in regulating the phenotypic expression of *Mp*, *vao*, and *trf* genes.

SUMMARY

The transformer (*trf*) gene modifying all floral parts, rendering the flower sterile, exerts a powerful activating and deactivating influence on a factor controlling maroon pigmentation and orange variegation (*vao,* vao^R) genes located in its neighborhood on the same stretch of DNA. The activation of these genes, including the *trf* itself, is conditioned by the methylation status of the activator (*Act*) gene, which is linked with the *vao-trf* group, and by an independent transposable element (*Tel*) which shifts from the nonfunctional (Tel^n) to the active (Tel^a) form with variable frequencies, depending on the

level of the developmental phase in which the specific section of the shoot happens to be. Unequal hampering of any step in the process of methylation of DNA of the *vao-trf-Act* segment along the growing shoot, may be the basis for a disproportional distribution of individuals in phenotypic classes of cultures grown from seeds collected at different levels (nodes) of the stem – that is why, in genetic testing neither statistically acceptable typical segregation ratios for transformed (*trf*) vs. revertant (*trf*^{r}) phenotypic classes, nor reliable crossing-over values for the genes involved, could be obtained.

Transcription and conversion of the nonfunctional transposable element (*Tel*^{n}) to the active form *Tel*^{a}, the transposition of the latter, as well as the methylation of DNA of the *vao-trf-Act* segment appear to be affected by the developmental phase changes, conditioned, apparently, by the DNA methylation status of regulator genes. Because the active transposable element *Tel*^{a}, which predisposes DNA methylation of the *vao-trf-Act* segment, is produced from nonfunctional *Tel*^{n} in mitotically dividing cells of the somatic tissue, the variants – maroon-leaved adult plants (*Mp*), green-leaved orange plants (*vao*^{t},*vao*^{tR}), and plants with transformed (*trf*) as well as wild-type revertant (*trf*^{r}) flowers – may be referred to as heritable soma-controlled traits.

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Table 1. Segregation in progenies of self-fertilized and intercrossed maroon-leaved transformed (*trf*) mutants of *C. heterophylla*.

Cultures	Plant	Phenotype	Parent(s) phenotype	Revertant *trf*^{r}, green	Transformed *trf*, maroon	Total
h9382,3	h9247-1	*trf*^{6}	*Trf*/*trf*	10	4	14
h94251	h9382-3	*trf*^{12}	*trf*^{6}	20	7	27
h94256	h9383-9	*trf*^{10}	*trf*^{6}	6	9	15
h95146,7,8	h94252-1	*trf*^{5}	*trf*^{10}x*trf*^{12}	5	64	69
h96238,9	h95119-3	*trf*^{8}	*trf*^{7}x*trf*^{8}	8	3	11
243	119-6	*trf*^{5}	"	23	20	43
249	h95126-1	*trf*^{5}	*trf*^{r}x*trf*^{10}	10	15	25
253	126-15	*trf*^{3}	"	5	8	13
256	126-20	*trf*^{3}	*trf*^{r}x*trf*^{r}	11	10	21
265	h95137-7	*trf*^{5}	"	2	8	10
270	137-8	*trf*^{13}	"	21	8	29
271	137-19	*trf*^{4}	"	8	16	24
277	h95141-7	*trf*^{4}	"	1	23	24
281,2,3	h95146-6	*trf*^{5}	*trf*^{5}	23	77	100
284	146-9	*trf*^{6}	"	9	10	19
286	146-11	*trf*^{2}	"	3	27	30
287	146-18	*trf*^{3}	"	2	18	20
288	146-23	*trf*^{2}	"	4	24	28
293	146-31	*trf*^{1}	"	0	8	8
295	146-33	*trf*^{5}	"	11	9	20
299	h95148-13	*trf*^{3}	"	12	25	37
301	148-20	*trf*^{4}	"	9	23	32
305	148-30	*trf*^{2}	"	5	48	53
307	148-32	*trf*^{cot*}	"	0	11	11
308	148-46	*trf*^{4}	"	0	39	39
315	h95153-5	*trf*^{2}	*trf*^{10}x*trf*^{5}	0	9	9
316	153-7	*trf*^{6}	"	9	3	12
317	153-9	*trf*^{7}	"	13	6	19
h97161	h96302-13	*trf*^{3}	*trf*^{r}x*trf*^{2}	3	12	15
163	303-19	*trf*^{1}	"	7	17	24
165	h96305-2	*trf*^{2}	*trf*^{2}	0	19	19
166	305-7	*trf*^{3}	"	7	16	23
170,1	h96306-15	*trf*^{1}	*trf*^{2}x*trf*^{2}	5	11	16
177	h96308-24	*trf*^{2}	*trf*^{4}	2	23	25
h98367	h96253-10	*trf*^{3}	*trf*^{3}	0	10	10
387	h96317-9	*trf*^{2}	*trf*^{7}	1	10	11
h94252,3	h9382-3x83-9	*trf*^{12}x*trf*^{10}	*trf*^{6}	8	9	17
h96242	h95119-5x119-6	*trf*^{12}x*trf*^{5}	*trf*^{7}x*trf*^{8}	7	16	23
285	h95146-9x146-6	*trf*^{6}x*trf*^{3}	*trf*^{5}	5	10	15
289	146-23x146-6	*trf*^{2}x*trf*^{3}	"	1	6	7
292	146-28x146-9	*trf*^{2}x*trf*^{6}	"	6	33	39
294	146-31x148-1	*trf*^{1}x*trf*^{2}	"	0	18	18
306	h95148-31x148-1	*trf*^{2}x*trf*^{2}	"	10	27	37

* cot = cotyledon node

Table 2. Segregation in progenies of self-fertilized and intercrossed phenotypically wild-type revertant, *trf*^{r}, mutants of *Collinsia heterophylla*.

Cultures	Plant	Phenotype	Parent(s) phenotype	Revertant *trf*^{r}, green	Transformed *trf*, maroon	Total
h94152	h9383-5	*trf*^{r}	*trf*^{6}	12	0	12
h94258	h9385-8	"	"	14	0	14
h95131	h94253-1	"	*trf*^{12}x*trf*^{10}	33	0	33
h96250	h95126-2	"	*trf*^{r}x*trf*^{5}	21	0	21
h97179	h96310-17	"	*trf*^{10}x*trf*^{5}	18	0	18
h98351	h96191-15	"	*trf*^{r}x*trf*^{4}	33	0	33
Totals				**131**	**0**	**131**
h95121	h94249-3	*trf*^{r}	*trf*^{r}x*trf*^{10}	28	3	31
129	250-9	"	*trf*^{r}x*trf*^{4}	56	6	62
132	253-1x253-12	*trf*^{r}x*trf*^{r}	*trf*^{10}x*trf*^{10}	32	4	36
138	253-12	*trf*^{r}	"	27	3	30
h96255	h95126-18	"	*trf*^{r}x*trf*^{12}	20	1	21
272	137-16	"	*trf*^{r}x*trf*^{r}	20	2	22
280	141-10	"	"	20	1	21
462	376-2	"	"	40	6	46
463	376-7	"	"	27	2	29
464	376-10	"	"	46	2	48
h97169	h96306-12	"	*trf*^{2}x*trf*^{2}	27	1	28
Totals				**343**	**33**	**376**
h95127	h94250-1	"	*trf*^{r}x*trf*^{4}	19	6	25
143	254-4	"	*trf*^{7}x*trf*^{10}	29	11	40
151	253-2	"	*trf*^{10}x*trf*^{10}	17	3	20
h96240	h95119-4	"	*trf*^{7}x*trf*^{7}	14	6	20
252	126-11	"	*trf*^{r}x*trf*^{12}	18	3	21
262	137-3	"	*trf*^{r}x*trf*^{r}	19	3	22
267	137-12	"	"	12	4	16
269	137-16	"	"	40	13	53
273	137-31	"	"	21	3	24
310	h95149-3	"	*trf*^{10}x*trf*^{5}	20	3	23
h97178	h96309-9	"	*trf*^{r}	18	4	22
Totals				**227**	**59**	**286**
h95134	h94253-6x253-14	*trf*^{r}x*trf*^{r}	*trf*^{10}x*trf*^{10}	18	11	29
135	253-9	*trf*^{r}	"	18	15	33
137	253-11x253-12	*trf*^{r}x*trf*^{r}	"	11	16	27
139	253-14	*trf*^{r}	"	16	15	31
140	h94254-1x254-3	*trf*^{r}x*trf*^{r}	*trf*^{5}x*trf*^{10}	16	15	31
141	254-1x254-6	"	"	4	7	11
142	254-3	*trf*^{r}	"	19	10	29
145	254-3	"	"	27	21	48
h96240	h95119-7	"	*trf*^{r}x*trf*^{7}	41	20	61
261	136-24	"	*trf*^{r}	9	7	16
h97159	h96301-6x301-7	*trf*^{r}x*trf*^{r}	*trf*^{4}	9	7	16
160	301-7	*trf*^{r}	"	9	5	14
Totals				**197**	**149**	**346**
h95136	h94253-11	*trf*^{r}	*trf*^{10}x*trf*^{10}	6	21	27
h96257	253-11	"	"	19	24	43
296	h95146-35	"	*trf*^{8}	7	23	30
Totals				**32**	**68**	**100**

Table 3. Segregation in progenies of self-fertilized green revertant *trf*^{r} plants and crosses between revertant (*trf*^{r}) and transformed (*trf*) mutants of *Collinsia heterophylla*.

Cultures	Plants	Phenotypes	Parents' phenotypes		Revertant *trf*^{r} green	Transformed *trf*, maroon	Total
h94249	h9382-1x83-9	*trf*^{r}x*trf*^{10}	*trf*^{6}		6	9	15
250	82-1x207-13	*trf*^{r}x*trf*^{4}	*trf*^{4}		13	3	16
259	85-8x85-7	*trf*^{r}x*trf*^{10}	*trf*^{5}		11	0	11
h95122	h94249-3x252-1	*trf*^{r}x*trf*^{5}	*trf*^{r}x*trf*^{9}	*trf*^{12}x*trf*^{13}	4	6	10
123	249-5x252-1	*trf*^{r}x*trf*^{5}	*trf*^{r}x*trf*^{9}	*trf*^{12}x*trf*^{13}	19	7	26
124	249-13x250-12	*trf*^{r}x*trf*^{10}	*trf*^{r}x*trf*^{9}	*trf*^{r}x*trf*^{10}	16	10	26
125,6	249-13x252-1	*trf*^{r}x*trf*^{5}	*trf*^{r}x*trf*^{9}	*trf*^{12}x*trf*^{13}	12	38	50
128	h94250-4x250-1	*trf*^{12}x*trf*^{r}	*trf*^{r}x*trf*^{4}		22	5	27
130	250-9x250-12	*trf*^{r}x*trf*^{12}	*trf*^{r}x*trf*^{10}	*trf*^{r}x*trf*^{10}	6	1	7
133	253-6x253-10	*trf*^{r}x*trf*^{10}	*trf*^{12}x*trf*^{10}	*trf*^{12}x*trf*^{10}	12	7	19
144	254-6x250-12	*trf*^{r}x*trf*^{12}	*trf*^{5}x*trf*^{10}	*trf*^{r}x*trf*^{10}	23	13	36
150	253-4x253-2	*trf*^{12}x*trf*^{r}	*trf*^{12}x*trf*^{10}	*trf*^{12}x*trf*^{10}	15	2	17
152	254-2x254-6	*trf*^{8}x*trf*^{r}	*trf*^{5}x*trf*^{10}	*trf*^{5}x*trf*^{10}	1	6	7
153	256-7x252-1	*trf*^{r}x*trf*^{5}	*trf*^{10}	*trf*^{12}x*trf*^{10}	2	27	29
h96245	h95119-7x119-6	*trf*^{r}x*trf*^{6}	*trf*^{7}x*trf*^{8}	*trf*^{7}x*trf*^{8}	2	7	9
246	122-9x122-8	*trf*^{r}x*trf*^{9}	*trf*^{r}x*trf*^{5}	*trf*^{r}x*trf*^{5}	6	7	13
260	136-12x136-15	*trf*^{3}x*trf*^{r}	*trf*^{r}	*trf*^{r}x*trf*^{r}	4	15	19
274	137-31x137-18	*trf*^{r}x*trf*^{10}	*trf*^{r}x*trf*^{r}	*trf*^{r}x*trf*^{r}	7	1	8
302	148-25x146-11	*trf*^{r}x*trf*^{2}	*trf*^{5}	*trf*^{8}	5	15	20
303	148-25x148-30	*trf*^{r}x*trf*^{2}	*trf*^{5}	*trf*^{8}	8	20	28
309	h95149-1	*trf*^{r}	*trf*^{10}x*trf*^{5}		7	3	10
310	149-3	*trf*^{r}	*trf*^{10}x*trf*^{5}		20	3	23
314	151-23x268-24	*trf*^{13}x*trf*^{r}	*trf*^{r}	*trf*^{r}	17	0	17
319	153-10x153-17	*trf*^{r}x*trf*^{5}	*trf*^{7}x*trf*^{8}	*trf*^{7}x*trf*^{8}	25	3	28
320	153-10x153-26	*trf*^{r}x*trf*^{8}	*trf*^{7}x*trf*^{8}	*trf*^{7}x*trf*^{8}	17	6	23
321	153-10x153-24	*trf*^{r}x*trf*^{8}	*trf*^{7}x*trf*^{8}	*trf*^{7}x*trf*^{8}	9	8	17
322	h95153-10x196-21	*trf*^{r}x*trf*^{4}	*trf*^{7}x*trf*^{8}	*trf*^{r}	12	1	13
h97179	h96310-17	*trf*^{r}	*trf*^{10}x*trf*^{10}	*trf*^{10}x*trf*^{5}	18	0	18
h98149	h96237-6x253-9	*trf*^{r}x*trf*^{10}	*trf*^{7}x*trf*^{5}	*trf*^{8}	10	1	11

Figures 1-6. Transformed, *trf*, mutants of *Collinsia heterophylla*.

Fig. 1. Wild-type with maroon patches on juvenile leaves.

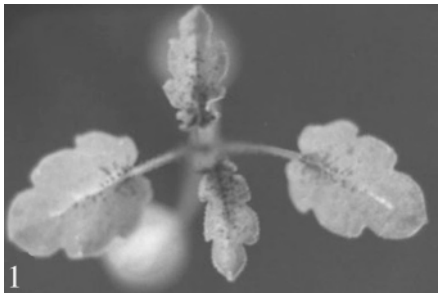


Fig. 2. Wild-type, normal flowers with maroon dots.

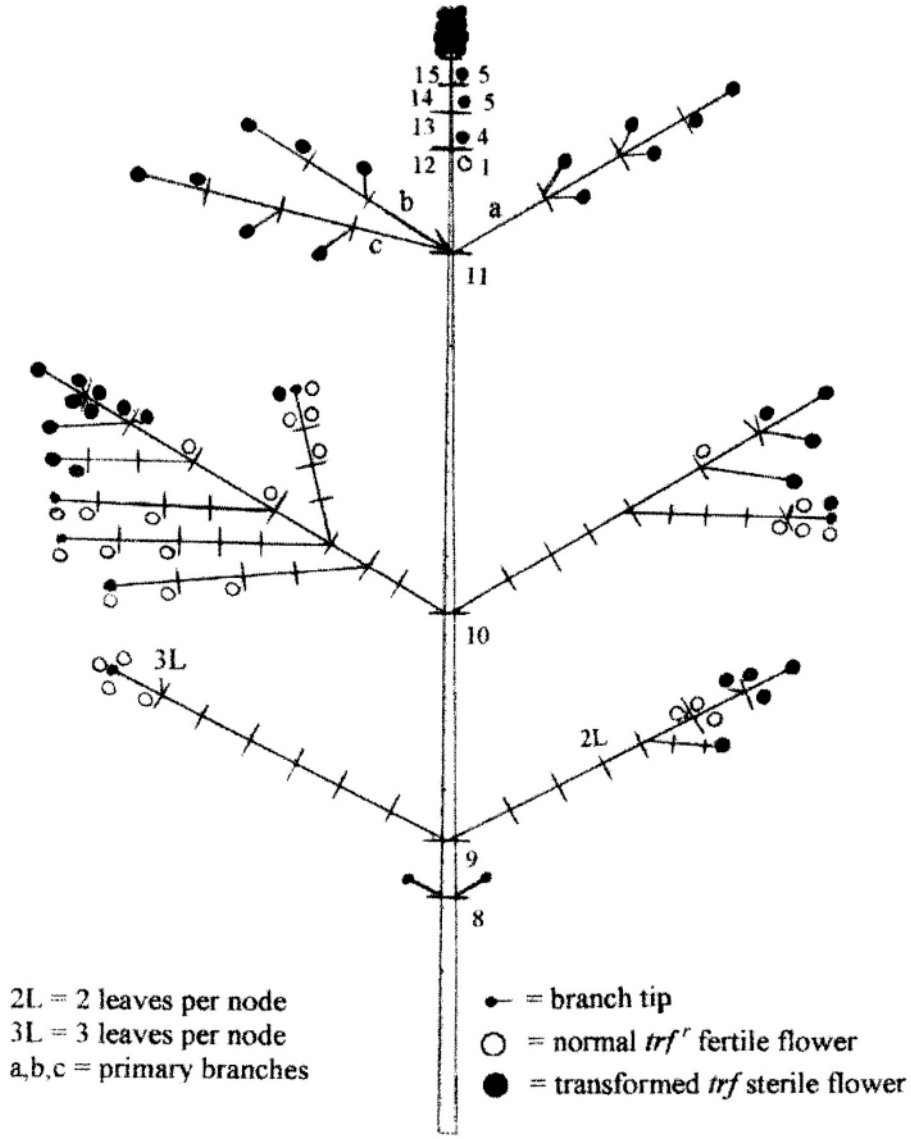
Fig. 3. Transformed, *trf*: leaves maroon from 4^{th}, 6^{th} node up.

Fig. 4. Transformed, *trf*,: "cone" with "incomplete flowers."

Fig. 5. Transformed, *trf*: sterile transformed (*trf*) flowers.

Fig. 6. Transformed, *trf*: branch with *trf* flowers (left), branch with *trf'* flowers (right).

Figure 7. Reproductive maturation level and transformation of flowers in transformed (*trf*) mutant (h9383-9) in *Collinsia heterophylla*.



Figures 8-13. Transformed, *trf*, and orange variegation (*vao, vao*^{R}) mutants of *Collinsia heterophylla*.

Fig. 8. Transformed, *trf*: maroon leaves at 2nd, 3rd nodes up.

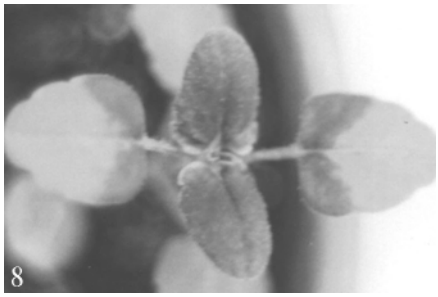


Fig. 9. Transformed, *trf*: maroon leaves at 11th node and up.

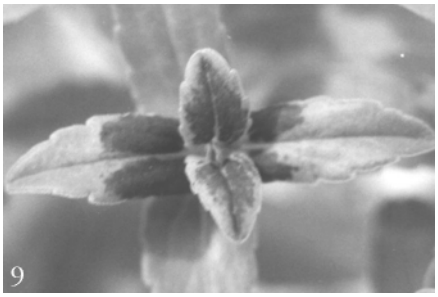


Fig. 10. Transformed, *trf*: maroon bracts in reproductive phase.

Fig. 11. Transformed, *trf*: orange, *va*o: from 3rd node leaf and up green, *vao*^{t}.

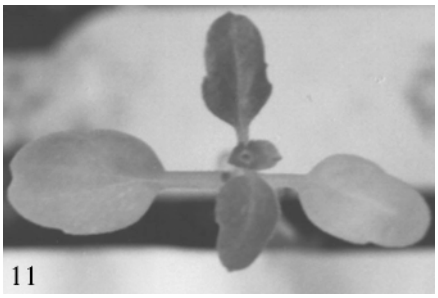


Fig. 12. Transformed, *trf*: 2nd node leaf orange, *vao*; 3rd light green, 4th dark green (3rd, 4th *vao*^{t}).

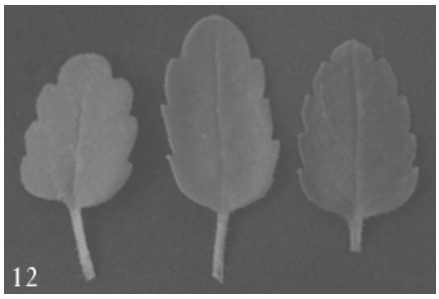
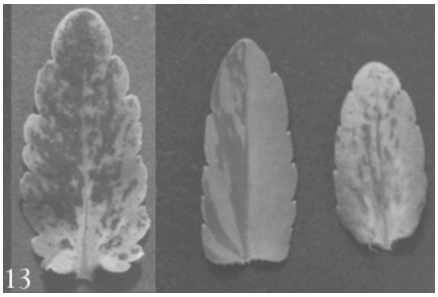


Fig. 13. Leaves, left to right: maroon, orange green *vao*^{R}, transformed orange *vao*^{t}.



Figures 14-19. Phenotypes of *Collinsia* plants:
Figs. 14-17. *C. heterophylla*, Figs. 18-19. *C. verna*.

Fig. 14. Transformed, *trf*: left to right, 4th node vao^R, 5th and 6th node leaf pairs vao^{tR}.

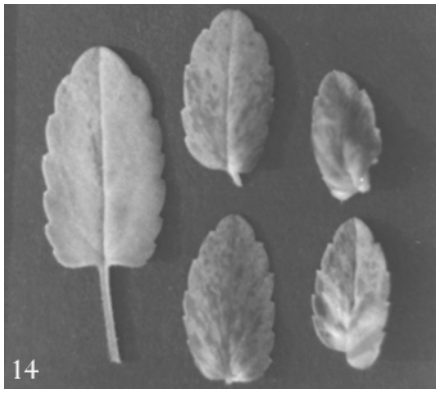


Fig. 15. Transformed, *trf*: left to right, 2nd node vao^R, 3rd and 4th node and up vao^{tR}.

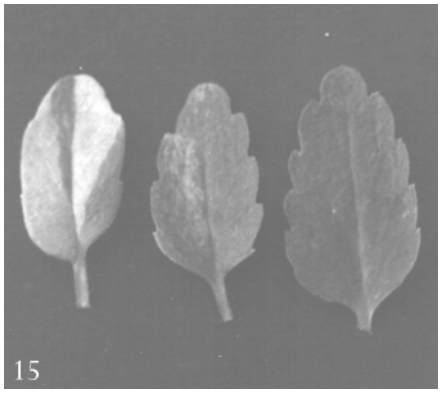


Fig. 16. Transformed, *trf*: lateral actinomorphic flowers, *trf/trf sf/sf* kl^p/kl^p *kn/kn* genotype.

Fig. 17. Left-upper lip: *Md, Uc, Ud, Ur*; middle and right – *Wf* keel flower formation.

Fig. 18. *C. verna*: leaves without and with maroon patches.

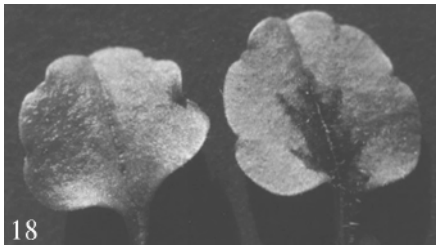


Fig. 19. *C. verna* flowers: keel fl.; without and with maroon.