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# A phylloid ground state of reverted floral specimens of *Psophocarpus tetragonolobus* (L.) DC (Fabaceae): Cancelled floral meristem and continued floral organ identity

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## Abstract

Recombinants of *Psophocarpus tetragonolobus* (L.) DC (Fabaceae), homozygous for a recessive allele of a master homeotic gene reverted from a normal photosynthetic, sexual reproductive nature to a vegetative, non-reproductive nature. This included transmutative transformation of floral meristems to a non-sexual phylloid floral ground state where the virescent organs maintained their identity but floral meristem identity was cancelled thus giving rise to a form of anachronic reversion. This was usually followed by a scenario of phyllotactic alterations involving the elongation of the floral axis which physically transformed flowers, in varying degrees of spatial permutations by the formation of ancestral floral structures, including gynophore and a pericladial stalk: a form of paleochronic reversion. Research verified that an allele of the master homeotic gene responsible for this phenomenon is a prerequisite to that scenario. Specific permutations are directly controlled by at least four additional homeotic genes recognized, defined and functionally characterized herein. Their qualitative functions (i.e. dominant or recessive) are responsible respectively for the carpel form, being either vascularized (*VSCARP*) or digonolobe (*vscarp*); the state of the gynophore being formed (*GNF*) or nascent (*gnf*); the state of the pericladial stalk being formed (*PCL*) or nascent (*pcl*) and the bracts position remaining parallel (*BCT:PRL*) at the calyx (or on the pericladial stalk) or being dislocated due to an interbractial stem formation between bracts (*bct:prl*). Results indicate that floral meristem identity once established can naturally be cancelled with little or no effect on floral organ identity.


***Keywords:*** Meristem cancellation; Organ identity; Permutation; Phyllotaxis; Paleochronic reversion; Transmutation

## Introduction

The developmental "ground state" (Coen and Meyerowitz, 1991) of flowers has been a point of inquiry for centuries. Goethe considered specific organs of the four floral whorls (i.e. sepals of the calyx, petals of the corolla, stamens of the androecium and carpel of the

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gynoecium) to be modified leaves (Surridge, 2004; Weigel and Meyerowitz, 1994), the flower thus being a "metamorphosed shoot" (Fahn, 1985) arising after evocation (i.e. transition) of the vegetative shoot meristem to a floral meristem (Battey and Lyndon, 1990; Okamuro et al., 1993). He postulated the possibility of the "…ideal basic organ" (Goto et al., 2001) to account for the ground-state floral organ. Receiving and surviving major challenges (Fahn, 1985), this postulation is verified by research (Ditta et al., 2004) that demonstrates floral meristem and organ reversion to a "leaf-like" (i.e. phyllome) ground state.

Floral reversion as treated here refers strictly to a phylloid condition where floral organs on completely or near completely induced flowers become virescent and the entire organ system becomes meristematically inactive (i.e. cancelled). It corresponds to Swartz's (1971) definition of reversion as "…the reappearance of an ancestral character not exhibited by the immediate parent…". Neither, one must add, does that ancestral character correspond to the immediate antecedent form and function (i.e. sexual reproduction) of the normal, non-reverted flower. This reversion coincides with Battey and Lyndon's (1990) observation that "…plants may bear flowers that have some vegetative characteristics…" (p. 165) including a "…range of possible structures from purely vegetative at one extreme to a complete inflorescence at the other, …" (p. 165) and "…the floral axis terminates with leaves or a leafy shoot" (p. 164). In this case, a modicum of "virescent vegetative" state can be observed as the resulting "leaves" are in reality virescent phylloid floral organs, already of determinate growth (Benya, 1995) and the floral apical meristems disappear (i.e. cease meristematic activity) or are completely lost (Kidner and Martienssen, 2005).

Based on homeotic gene expression, postulated origin, (Coen et al., 1990; Stewart and Rothwell, 1993) and theoretical morphologic transformation (Kramer and Irish, 1999), bracts or "floral leaves" (Coen et al., 1990; McLean and Ivimey-Cook, 1961; Stern, 1988) can be considered a fifth group of floral organs. These usually remain leafy or phylloid. However, wide morphologic variations are well documented for bracts (Stern, 1988; Tooke and Battey, 2000) as well as for other floral organs (Irish and Litt, 2005).

The term "transition" denotes the inductive metamorphosis, involving meristem identity genes, of an apical shoot vegetative meristem into a floral reproductive meristem (Battey and Lyndon, 1990; Okamuro et al., 1993; Parcy et al., 2002). The term "transformation" is more specifically applied to homeotic conversion or reversion. Homeotic conversion applies to any inductive transformation of a structure defined at one level to a higher and/or more specific level such as the conversion of cauline leaves to petals (Goto et al., 2001) or petals to stamens (Pautot et al., 2001). Homeotic reversion applies to a regressive transformation of a structure defined at a certain level to a lower and/or more general level such as the homeotic reversion metamorphosis of a floral meristem (Okamuro et al., 1996; Parcy et al., 2002) and/or floral organs (Goto et al., 2001; Honma and Goto, 2001; Theiβen and Saedler, 2001) to a shoot meristem with the accompanying phylloid or phyllome structures. However, transformation includes both transmutation and permutation. Transmutation denotes an essential change or revertive transformation in the nature of the meristem to a developmental or ancestral antecedent form (Swartz, 1971) as for example determinate, sexual reproductive function vs. indeterminate, non-reproductive, photosynthetic growth function (Benya, 1995, 1999, 2000; Parcy et al., 2002) or merely to a phylloid floral state (Schwarz-Sommer et al., 1990). Permutation denotes morphologic phyllotactic alteration (i.e. spacing, organizational change or organ rearrangement) of an organism, organ system or organ. These modifications are due to mutated floral homeotic genes, which specify or misspecify organ identity causing "…misinterpretation of positional information in the developing flower and subsequent homeotic transformation of floral organ types." (Goto et al., 2001, p. 449), including heterochronic, that is spatial (Coen, 1991) and/or temporal (Ikeda et al., 2005; Kidner and Martienssen, 2005) reversion or transformation (Goto et al., 2001; Ikeda et al., 2005). The term "distance", as used by McLean and Ivimey-Cook (1961), is preferred to the more general terms of "space" or "spacing" because of the continuity of established usage and because it conveys more accurately the sense of structural morphogenesis reported herein.

The ABC genetic model (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994) with continuing (e.g. DE) modifications (Honma and Goto, 2001; Jack, 2004) is largely successful in explaining specification of organ identity in floral whorls that are normal, ectopic or permutated. Using this model and considering the homeotic genes associated with it Coen and Meyerowitz (1991) affirmed Goethe's supposition after studies of double and triple homeotic mutants in *Arabidopsis* whose transformed flowers consisted exclusively of "…organs resembling cauline leaves…" This research anticipated that of Ditta et al. (2004) using quadruple homeotic mutants of the same species which showed that established floral organ identity can change thus giving rise to floral organs totally transformed to complete phyllomes (i.e. "leaves"). All of these results can be considered as different types of "developmental ground states".

Research concerning specification of meristem and organ identities (Ditta et al., 2004; Goto et al., 2001; Theiβen, 2001) in angiosperm flowers has revealed a plethora of both meristem identity genes and homeotic

floral organ identity genes (Parcy et al., 1998), both epigenetic (Amedeo et al., 2000; Cubas et al., 1999) and non-epigenetic (Coen et al., 1990; Weigel and Meyerowitz, 1993, 1994), some with redundant effects specifying both meristem and organ identities (Ditta et al., 2004; Okamuro et al., 1993; Parcy et al., 1998). These genes are capable not only of permutating the spacing, order and arrangement of floral organs (Goto et al., 2001; Pelaz et al., 2000) as witnessed by ectopic organ expression, but also of transmutating already identified, virescent organs (e.g. leaves) to "floral specific activity" (Honma and Goto, 2001). Conversely, meristematically identified flowers have been transformed "…into leaves with associated shoots…" (Parcy et al., 1998). Floral meristem identity can even be lost (Kidner and Martienssen, 2005).

A homeotic recessive, non-epigenetic gene (Benya, 1995, 1999) has been recognized and, at least partially, "functionally characterized" (Ripoll et al., 2006) in recombinant specimens of the species *Psophocarpus tetragonolobus* (L) DC (Fabaceae). Specimens homozygous for the gene in its activated state, field grown in the northeast region of tropical equatorial Brazil (i.e. Teresina, Piaui) presented an anachronic "reversion" morphology. Organs of three of the four floral whorls (i.e. petals, stamens and carpels), having developed organ identity showed some degree of anachronic reversion (i.e. return or regression) to a non-sexual, phylloid ground state morphology (Fig. 1 and Supplementary material) because the specified, floral meristem activity was cancelled (i.e. completely inactivated). Characteristics of this state also included virescence of the whorl organs and in the case of the tetragonolobe conduplicate pod (Fig. 2A) and its normal antecedent digonolobe carpel (Fig. 2B), maintenance of the digonolobe or flat conduplicate form in a reverted state (Fig. 2C) (Benya, 1995). Reversion, once initiated was qualitatively variable within a population and even

**Fig. 1.** Normal flower (right): pigmented petals, sexual reproductive function. Reverted flower (left): transmutated, virescent, phylloid organs, cancelled meristem.

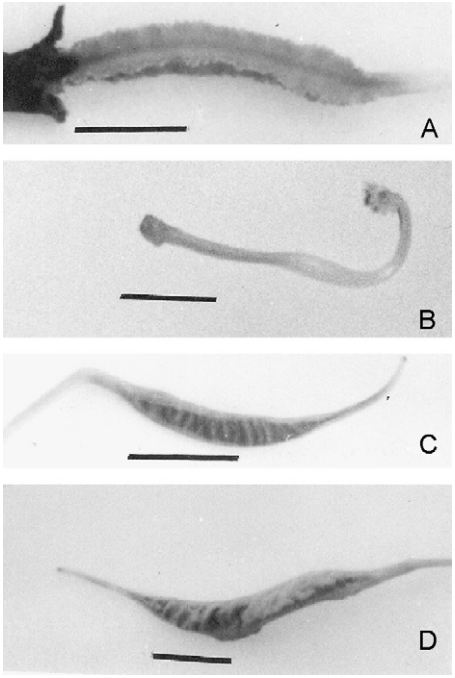


**Fig. 2.** (A) Conduplicate (tetragonolobe) pod, (normal), (B) conduplicate (digonolobe) carpel, (normal), (C) Conduplicate (digonolobe) carpel (reverted) phylloid ground state, (D) Conduplicate (vascularized) carpel (reverted), deformed.

within sections of a component plant (i.e. recombinant) of that population. Reversion showed significant linear correlation with age of individual recombinants and with different environmental variables, including "mean monthly evaporation" and "mean daily range in temperature" (Benya, 1995, 1999) thus diminishing the possibility of 100% (*de novo*) reversion each generation in any population.

A frequent, rather rapid and fairly systematic transformation of most phylloid ground state flowers to various phyllotactically altered forms further suggested the presence of more than one homeotic gene, involved in this reversion phenomenon (Benya, 1995, 1999). The purpose of the present research was to document phenotypic (genetically functional) characteristics associated with the phylloid ground state reverted flowers of this species as well as any associated morphologic states, their possible sequence(s) of occurrence and any analogies to/with ancestral angiosperm forms.

## Material and methods

Plantings of naturally reverted recombinants of *P. tetragonolobus* (L) DC, field grown in the tropical equatorial, semiarid environment of Teresina, Piaui, Brazil (05°05′S; 42°49′W, alt. 64 m) produced both reverted and normal floral specimens. Recombinants

were defined by their homozygous constitution for the recessive allele of the master gene responsible for the reversion phenomenon. Reversion had already been noted for individual plants. Reverted carpels did form, but their capacity to produce seed was completely cancelled. Proportions of reversion rate within any population were thus crucial. A group of 74 non-reverted plants (termed P1) was allowed to self-pollinate, a phenomenon which naturally approaches 100% (even with the presence of bees) for this species (Khan, 1982). Sample P1 plants of this group furnished seed that was isolated and individually referenced to its P1 parent. If the P1 then reverted, its isolated seed was further documented as having reversion capacity. Reversion rate for the P1 group (i.e. 18 of 74 plants or 24.32%) significantly ($\chi^2 = 0.018$; $p < 0.001$) approached the ideal 25% or 1:3 phenotypic ratio indicative of a single gene (two allele) recessive:dominant effect. Seed from reverted P1 plants was mixed and planted yielding an F1 generation, a sample of which ($n = 14$) showed 13 reversions, a frequency of 92.86%. Seed of this population (and all other populations subjected to this same procedure whose reversion rate was 90% or more) was defined as ''recombinant homozygous recessive'' for the master gene isolated herein. Thus allele recognition and definition are based on phenotypic (i.e. anatomic and morphologic) analysis of individual recombinant plants and statistical analysis of samples within plant populations showing reversion and associated morphogenesis; at least a partial ''functional characterization'' (Ripoll et al., 2006) of genes.

Reverted floral specimens originating from various recombinants (including repetitions from specific recombinants) were examined directly in the field or gathered and maintained in plain or deionized water and then examined. A total of 338 previously examined specimens furnished data concerning cancelled floral meristems while another 284 provided information as to digonolobe or tetragonolobe morphologic forms (Benya, 1999). A random group of 141 floral reverted specimens yielded data for presence of any phyllotactic alteration while 25 of these 141 presented specific reference of phyllotactic carpel alterations within the phylloid ground state. A subset (i.e. 24 of these 25 specimens) yielded further data concerning specific permutations with respect to morphogenesis of the gynophore, pericladial stalk and interbractial stem.

## Results

By definition and through their essential reproductive potential, all floral buds prior to reversion had received meristem identity and floral organ identity specified by meristem identity genes and ABCDE organ identity genes, respectively. These buds then reverted from a sexual reproductive function to a non-sexual photosynthetic function; a phylloid floral state. They underwent a transmutative transformation in floral nature where meristem reproductive identity was cancelled. Floral organs became phylloid or ''leaf like'' in form without a complete reversion to a ''phyllome'' form; not yet completely ''modified leaves''. Reversion brought a toughening of otherwise fragile organ tissue (especially petals, stamens and carpels) accompanied or preceded by virescence (Figs. 1 left, 2C, D and 3). Sepals and bracts maintained the virescence, which is normal to their specified identity.

Virescence of petals, stamens and carpels was the most obvious phenotypic indicator or morphologic sign of reversion to this phylloid state (i.e. the transmutative stage), occurring in 336 of 338 (99.4%) specimens (Fig. 1 left and Supplementary material). The variation therein arose at the petal region. Digonolobe carpel form (Figs. 1 left and 2C); however, was the most consistent morphologic sign of this stage, occurring in 284 of 284 (100%) specimens examined while cancellation of floral meristem identity was the most consistent functional sign of this stage. All three changes, in fact, are keys to the recognition and documentation of the phylloid floral ground state. The reverted flower at this stage is transmutated in essence (i.e. sexual reproductive vs. non-sexual phylloid). However, it is not permutated, either spatially or organizationally and could remain as such showing no further morphologic change. Only buds that have undergone complete evocation (i.e. completely florally induced) should remain at this stage.

A permutative stage usually followed this transmutative stage on most floral specimens (97.16% of samples studied). A variable but predictable series of events usually occurred. A succession of sites could arise where permutative transformation, especially spatial alteration, reflected morphologic activity leading to major phyllotactic alterations of the reverted floral axis. Such alterations could be terminal, marking an end to any further phyllotactic alteration or they could be preliminary to further alterations.

No single morphologic alteration uniquely marked the initiation of the permutative stage, as did the three key alterations cited above for the transmutative stage. However, a sample of 34 floral specimens presented 33 (97.06%) individuals with a developed (or developing) gynophore that distanced the carpel or the fourth floral whorl anatomic region from stamens and all other preceding floral anatomic regions (Fig. 3). No other permutative characteristic reached this degree of presence although vascularization of the carpel (Fig. 2D) approached it in 31 of 34 (91.18%) floral specimens sampled. Gynophore formation thus came closest to being the key (almost unique) defining character of the permutative stage. It could reach a total measured

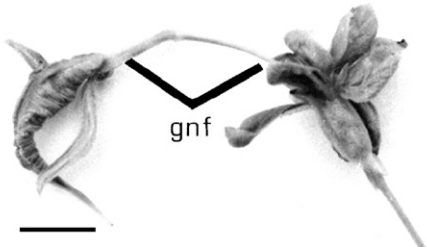


**Fig. 3.** Reverted flower with gynophore (*gnf*), center, composed of one node, two internodes phyllotactically distancing the carpel region (left) from the preceding three floral whorls (i.e. stamen, petal and sepal) regions and the pre-whorl bracts region (right); beginning phylloidy (i.e. foliation) of vascularized carpel (i.e. putative ovules).

length of 25 mm, composed of one node and two internodes of 15 and 10 mm, respectively.

An already vascularized carpel (Figs. 2D and 4) might then progress to some degree of foliation (Fig. 3). Occasionally, this included a complete "deadnation" probably along the adaxial carpel cleft resulting in an ovate phyllome that presented distinct pinnate venation (Fig. 5). On rare occasions, this phyllome could present numerous leaflets or putative phylloid ovules of both cauline and ovate form extending from its margins (Fig. 5).

A second possible site of permutation was at the floral base. The bracts region was physically distanced from the entirety of the four whorls regions (i.e. calyx or sepals and all succeeding regions) as a pericladial stalk formed (Fig. 4) that could reach a length of 70 mm. This stalk always took the form of an internodal extension of the calyx, distancing these organs from the first bract. When it did form, it usually succeeded (19 of 20 samples) or might be concomitant with the development of the gynophore. It is herein named "pericladial stalk" rather than complete pericladium because although this structure appears to be the distal part of the pedicellus, it lacks the constriction that would define it as a complete pericladium (McLean and Ivimey-Cook, 1961). Lacking this constriction suggests that it is an extension of the calyx or entire perianth rather than a complete pericladium (Fig. 4).

A third region of permutative transformation might arise at the bracts, normally positioned beneath the calyx, or on the pericladial stalk, at loci opposite each other (Fig. 6A). Each bract became rearranged, morphologically distanced or dislocated from the other when an interbractial stem developed (Fig. 6B). This might occur prior to, during, or after formation of the pericladial stalk.

Most floral changes occurred in the field on reverted flowers of reverted recombinants within hours after onset of reversion. They gave reverted floral specimens, a form referential to a more primitive ancestral

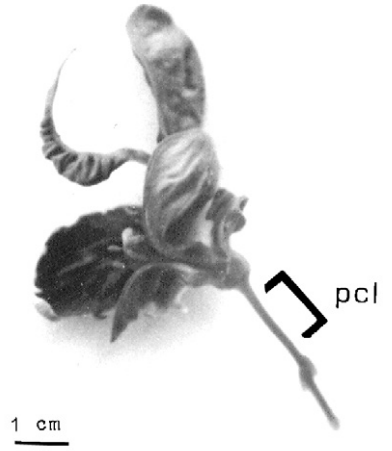


**Fig. 4.** Reverted flower (virescent organs) with partially shown gynophore (center) and fully developed pericladial stalk (pcl) about 12 mm long composed of one internode (bottom right) distancing the pre-whorl bracts region from the four floral whorls regions. The internode distancing both bracts has begun to form.

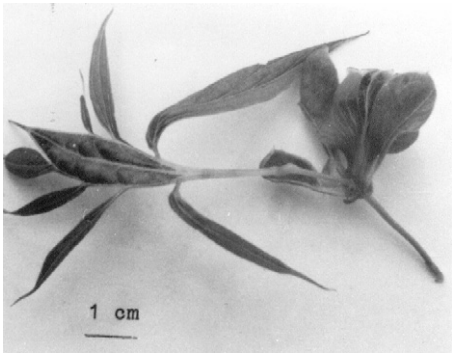


**Fig. 5.** Reverted flower: virescent organs, complete phyllome (thoroughly phylloid carpel) including putative phylloidic ovules of cauline (top and bottom) and ovate (extreme left) form on gynophore composed of one node, two internodes (center).

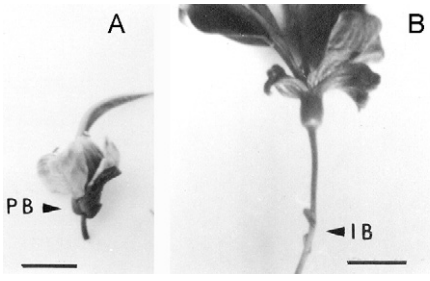


**Fig. 6.** Reverted floral specimens: (A) transmutated; a "phylloid ground state", PB = "Parallel Bracts", (B) transmutated and permutated, formed pericladial stalk, IB = inter bractial stem, about 4 mm.

morphology both "extant" (Friedman, 2006) and "extinct" (Stewart and Rothwell, 1993), based on organ regional distancing, phyllome venation, form and margins, and an appearance more like the vegetative shoot.

The recessive allele of the homeotic gene herein termed "*sexual reproductive state*" (*srs*), is an allele of a master gene whose dominant allele "*SEXUAL REPRODUCTIVE STATE*" (*SRS*) permits development of a plant whose flowers present the sexually reproductive state. The recessive allele, homozygous and activated in a recombinant, negates that development by cancelling (i.e. nullifying) established floral meristem identity. This master gene is essential to analysis and understanding of the present data, which indicate presence and activity of at least four additional genes whose qualitative effects can all function individually or in combination within the reverted recombinant.

The first of these genes, herein labeled "*VASCULARIZED CARPEL*" (*VSCARP*), affects carpel form in reverted recombinants. Its recessive allele (*vscarp*), in homozygous recombinants, results in the ground state of the digonolobe conduplicate carpel remaining as such. Its dominant allele (*VSCARP*), in the activated state, results in formation of the vascularized carpel. Statistical analysis of the sample of 24 reverted floral specimens (Table 1) showed a 16:8 dominant recessive distribution, reasonably close ($\chi^2 = 0.8888$, NS; Table 2) to the hypothetical 3:1 expected distribution. Such statistical analysis at this point, however, may lack the confidence it would show in non-reverted recombinants because of the previously mentioned qualitative uncertainty of the reversion phenomenon both between recombinants within the same environment and within parts of the same recombinant.

The second and third genes also demonstrated distinct dominant and recessive effects. Recessive alleles of both genes leave the reverted floral specimen at the phylloid ground state (Fig. 1, left). The dominant allele of the second gene, herein labeled "*GYNOPHORE*" (*GNF*) effects morphogenesis of a nascent gynophore (Fig. 3). Statistical analysis of a sample of 24 reverted floral specimens presented a 19:5 dominant:recessive distribution, reasonably close ($\chi^2 = 0.2223$, NS; Table 2) to the 3:1 expected distribution.

The dominant allele of the third gene, herein labeled "PERICLADIAL STALK" (*PCL*) effects morphogenesis of a pericladial stalk, which distances the bracts from all four floral whorls (Fig. 4). Statistical analysis of a sample of 21 reverted floral specimens presented a 16:5 dominant:recessive distribution, reasonably close ($\chi^2 = 0.0159$, NS; Table 2) to the 3:1 expected distribution.

The fourth of these genes herein labeled "*PARALLEL:BRACTS*" (*PRL:BCT*) affects the bracts, normally at parallel and opposite positions. The recessive (*prl:bct*) activated allele of this gene, in homozygous recombinants, permits development of a stem of internodal structure between the two bracts (Fig. 6B). A sample of 28 specimens from the original 141 showed a distribution of 19 having closed or parallel (i.e. normal) bracts (*PRL:BCT*) and nine having dislocated or distanced (*prl:bct*) bracts, reasonably approaching ($\chi^2 = 0.7619$, NS; Table 2) the expected 3:1 distribution indicative of a clear, dominant:recessive effect, but skewed toward specimens advanced into the reversion phenomenon. Distancing between bracts could occur with or without gynophore or pericladial stalk development on the same specimen. Conversely, gynophore and/or pericladial stalk development could

**Table 1.** Reverted flower: scenario of phyllotactic alterations, individual and combinations among the sample of 24 specimens

| Reverted floral condition | Carpel form | | |
|---|---|---|---|
| | Digonolobe | Vascularized | Sum |
| "Phylloid ground state" (five organ regions are continuous) | | | |
| (a) Parallel bracts | 4 | 0 | 4 |
| (b) Bracts separate | 0 | 1 | 1 |
| Gynophore present (carpel is distanced from stamens and all preceding regions) | | | |
| (a) Parallel bracts | 0 | 2 | 2 |
| (b) Bracts separate | 0 | 2 | 2 |
| Gynophore and pericladial stalk present (bracts region is distanced from whorls region and carpel is distanced from stamens, petals and sepals regions) | | | |
| (a) Parallel bracts | 1 | 1 | 2 |
| (b) Bracts separate | 2 | 4 | 6 |
| (c) Bracts indeterminate | 1 | 6 | 7 |
| Total | 8 | 16 | 24 |

**Table 2.** Phenotypic (morphologic/functional) characteristics, proportions and significance levels pertaining to genes and alleles involving the reversion scenario of samples of reverted floral specimens

| Phenotype | Allele(s) | Observed distribution | Expected distribution | $\chi^2$ | Significance |
|---|---|---|---|---|---|
| (1) Carpel form | | | | | |
| Vascularized | *VSCARP* | 16 | 18 | | |
| Digonolobe | *vscarp* | 8 | 6 | 0.8888 | NS |
| (2) Gynophore | | | | | |
| Present | *GNF* | 19 | 18 | 0.2223 | NS |
| Absent | *gnf* | 5 | 6 | | |
| (3) Pericladial stalk | | | | | |
| Present | *PCL* | 16 | 15.75 | 0.0159 | NS |
| Absent | *pcl* | 5 | 5.25 | | |
| (4) Bracts position | | | | | |
| Parallel | *PRL:BCT* | 19 | 21 | | |
| Separate | *prl:bct* | 9 | 7 | 0.7619 | NS |

occur without any stem formation between bracts. In a sample of 17 specimens showing dislocated bracts, 14 presented a pericladial stalk accompanying the interbractial stem, while three presented no pericladial stalk (i.e. one bract remained at the calyx while the second was some distance below it).

## Discussion

Digonolobe carpel form and meristem identity cancellation always occurred prior to bloom or opening of the reverted flower. Cancelled meristem identity (i.e. sexual sterility) was absolute. Sexual reproductive capacity terminated. Digonolobe carpel form, however, was morphologically referential but highly transient. Incomplete floral meristem "induction" (Battey and Lyndon, 1990; Benya, 1995) seemed to be the basis for this transience and prerequisite to any permutative effects of genes recognized herein. Vascularization of the carpel (Fig. 2D and Supplementary material) in the 25 referential sample specimens usually ensued. It seemed most common at the time of, or subsequent to formation of both gynophore and pericladial stalk (Table 1; Figs. 3 and 4). Rarely (two of 34 cases) it preceded or substituted both. Vascularization appears to be a prerequisite to any other phyllotactic alteration of the carpel including distinct phyllome formation (Fig. 5) and may result from incomplete floral organ specification and/or identification.

Reverted flowers present structures (e.g. reverted floral phylloid organs) that maintain floral organ identity but whose meristem identity is transformed (i.e. cancelled). The resulting phylloid organ system is neither root, nor inflorescence, nor pure floral but more like "flora vegetative" and is meristematically inactive.

At transmutation all meristematic cell division, meiotic and mitotic, terminates resulting in a phylloid ground state. At permutation mitotic cell division and/or cell expansion reappear at specific organ regions. Phyllotactic permutation through morphogenesis of nascent structures (e.g. gynophore development) is well documented in non-reverted legumes such as the peanut (*Arachis hypogaea*) as well as gynophore development on heterochronically reverted *sepallata* quadruple mutants of *Arabidopsis* (Ditta et al., 2004). The mitotic division that characterizes permutation of reverted flowers seems not to be of undifferentiated cells, but of already specialized cells at regions whose organ identity (e.g. gynophore and pericladial stalk) had probably been specified prior to reversion but whose development had remained nascent. The reverted organ system exhibits an orientation more toward indeterminate growth rather than the determinate growth characteristic of normal flowers.

Transformed floral buds and their phylloid floral organs present a new yet anachronous and distinct phenotype for possible regional permutative activity that can be characterized by a predictable scenario of qualitative events. These events increase in number and amplitude as their spatial and regional positioning from the calyx increases. The high degree of variability raises questions specific to the morphologic stability of the completely induced non-reverted flower (Battey and Lyndon, 1990; Okamuro et al., 1993), activation of each homeotic gene recognized herein, the effect of any further genes yet to be discovered, and the degree of canalization (i.e. homeostasis) (Okamuro et al., 1993) of effects. However, the predictability of effects indicates varying degrees of canalization, especially in the degree of floral evocation, particularly at the carpel.

The scenario usually begins and is qualitatively most extensive at the carpel and the carpel region. A symmetric reverted digonolobe conduplicate carpel (Fig. 2C) succeeds the symmetric pre-reversion (and

pre-pollination) conduplicate form (Fig. 2B). Virescence and toughening of tissue occurs about the same time. A gynophore can develop that distances the carpel from other floral organ regions. This is usually followed by vascularization, deformation and, at times, varying degrees of foliation of the organ. Stamens become virescent and tough at about the same time as the carpel undergoes this developmental pattern. Petals can become virescent and their tissue toughens, but the transformation (e.g. virescence) at this region seems more variable than in stamens and carpel. Sepals remain virescent and intact, the same as in the non-reverted flowers but their fused ''calyx'' form may give rise to clearly distinct cauline structures.

The bracts remain virescent just as in non-reverted flowers. However, bracts themselves can become distanced from each other as a generally short interbractial stem (e.g. 1–6 mm) develops (Fig. 6B). The entirety of the four floral whorls regions can become distanced from the pre-whorl bracts region as a pericladial stalk develops (Figs. 4 and 6B).

The tetragonolobe pod is a post organ identity form for the fertilized carpel. It probably results from pollination whose effect through the ovules (transformed into seeds) specifically and systematically transforms the symmetric digonolobe conduplicate carpel form (Fig. 2B) to a symmetric tetragonolobe pod in normal, non-reverted recombinants.

Pollination within the conduplicate carpel and succeeding fertilization of any ovules results in initiation of seed formation and accompanying organism identity ($2n$), in an embryonic state, of the next generation. Immature pods and seeds occurring on reverting recombinants can suffer complete cessation of development. Pods may become completely atrophied, as already reported (Benya, 1999). Such pods desiccate and die but never undergo the vascularization and foliation common to their carpel counterparts on the same reverted recombinant. Lack of pollination in the reverted ''flower'' seems sufficient to predispose the carpel (Fig. 2B) to permutative effects.

## Conclusion

The virescent, reproductively null, reverted flower, transmutated but not yet permutated, should be recognized as presenting a ''phylloid ground state'' whose transformed biologic nature is defined by three major characteristics: the digonolobe carpel; virescence and toughening of petal, stamen and carpel tissues, and cancelled meristem identity. It should be recognized as a ''metamorphosed flower'' showing continuity with the normal, sexually reproductive flower. It is thus a physiologically and metabolically functioning organ system having a cancelled meristem. Any stem cell activity in reverted flowers should have terminated prior to/or at reversion (Lenhard et al., 2001). However, this phylloid state is not yet the specific foliar (i.e. phyllome) ground state (Stewart and Rothwell, 1993) that Goethe postulated.

The permutative stage of reversion is herein termed the ''anachronous state'' because of the development of anatomic structures (e.g. gynophore) already present in non-reverted flowers (Fahn, 1985; McLean and Ivimey-Cook, 1961). It further defines and distinguishes reversion at the permutative stage as a ''paleochronic reversion'' rather than heterochronic (Goto et al., 2001; Ikeda et al., 2005; Parcy et al., 2002) or simple anachronic (spatial temporal) (Reddy and Meyerowitz, 2005; Tooke and Battey, 2000) because resulting organ systems and organs are referential to ancestral angiosperm organs that appear misplaced in time (Fahn, 1985; McLean and Ivimey-Cook, 1961).

The predictability of the initial transmutation plus succeeding permutative events indicates specific homeotic genes of non-epigenetic character. These genes always present morphologic effects (*de novo*) after activation of the master recessive allele (*srs*). Permutations follow a scenario indicative of homeotic gene activity which is similar to, yet distinct from that already documented in normal, non-reverted flowers.

A further four genes have been recognized here. Their names, alleles, action and effects, plus that of the master allele are summarized in Table 3. As with the recessive allele (*srs*) of the master gene whose activity is environmentally controlled (Benya, 1995) these four genes must also be considered non-epigenetic, environmentally controlled because their activity, when manifested, must always be manifest (*de novo*) each generation after activation of the *srs* allele. After gene activation, affected regions may then demonstrate the specific characteristics of permutation. This suggests that this entire reversion phenomenon may present a useful morphologic and even physiologic tool for use in paleobotany for comparative studies of these transformations and forms with those forms already identified in fossil and extant specimens of other angiosperm species both extant and extinct.

Specification of floral meristem identity and floral organ identities are distinct functions necessary for complete floral development in angiosperms. Concurrent transformation of both functions leading to the vegetative shoot and complete phyllome states respectively (Ditta et al., 2004) has confirmed Goethe's hypothesis that the flower is a metamorphosed shoot. Separation of these two functions within the groups of homeotic floral genes already identified and even as redundancy functions by certain of these genes (e.g. *lfy, AP1*) (Okamuro et al., 1993, 1996; Parcy et al., 1998) has allowed recognition of the distinct yet complementary relationship between these two specification functions.

**Table 3.** Genes and alleles associated with reversion and the scenario of phyllotactic alterations

| Trait | Gene name | Number of alleles | Allele | Action | Phenotype |
|---|---|---|---|---|---|
| Nature of function | *SEXUAL REPRODUCTIVE STATE* | 2 | *SRS* | Dominant | Recombinant is sexually reproductive |
| | | | *srs* | Recessive | Phylloid, non sexual, sterile recombinant |
| Carpel form | *VASCULARIZED CARPEL* | 2 | *VSCARP* | Dominant | Vascularized carpel |
| | | | *vscarp* | Recessive | Digonolobe carpel |
| Gynophore form | *GYNOPHORE* | 2 | *GNF* | Dominant | Formed gynophore |
| | | | *gnf* | Recessive | Nascent gynophore |
| Pericladial stalk form | *PERICLADIAL STALK* | 2 | *PCL* | Dominant | Formed pericladial stalk |
| | | | *pcl* | Recessive | Nascent pericladial stalk |
| Bracts Orientation | *PARALLEL BRACTS* | 2 | *PRL:BCT* | Dominant | Bracts at parallel loci |
| | | | *prl:bct* | Recessive | Bracts separated (i.e. inter bractial stem arises) |

The results presented here show that both functions can be separated not only theoretically and in laboratory studies (as already demonstrated), but also naturally in situ where floral meristem identity can be transmutated (e.g. cancelled) with little or no effect on floral organ identity. The resulting phylloid ground state is distinct from the phyllome ground state; both are forms of anachronic reversion. However, this phylloid ground state can also be preliminary to a possible paleochronic reversion where both phylloid and phyllome morphologic states can be manifest concurrently.

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## Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.flora.2006.09.004.

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