A Suite of Mutants that Modify Pattern Formation in Pea Leaves

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Modern molecular techniques offer unprecedented opportunities to explore the regulation of development in multicellular organisms. Still, classical embryology, anatomy, morphology and genetics retain their relevance. Indeed, the foundations for the current consuming interest in the homeotic mutants of Drosophila (Gehring and Hiromi, 1986; Fjose, 1986) and other organisms were laid through long years of classical genetic analysis (Lewis, 1978). Unusual importance is attached to homeotic mutants because they are postulated to regulate the activity of other genes involved in development. Increasingly, authors have emphasized the interdisciplinary approach to the problem of developmental regulation (e.g., Arthur, 1984; Stewart and Hunt, 1982; Wilkins, 1986).

Certain animals, among them the sea urchin, chick, frog, salamander, mouse, Caenorhabditis and Drosophila, are traditional experimental objects in developmental biology, Drosophila being especially favored, in part because of the extensive body of genetic and embryological literature accumulated on this organism over many years of intensive study. Plants have played a lesser role.

The scope of the present discussion is sharply circumscribed. Its single

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raison d'etre is to invite attention to a system of mutant genes which modify, often profoundly, a single organ, the leaf, of a single plant taxon, *Pisum*. This gene system is seen as favorable for investigating certain developmental processes at various levels, including the molecular level. Since it is primarily the spatial arrangement of already differentiated cells that is at issue in this review, the discussion concerns an aspect of development termed pattern formation. Much of the information relating to the actions and interactions of mutants within this system is found in publications which are neither well known nor readily accessible. Some of the mutants not only effect gross alterations in leaf morphology but also qualify as homeotic.

The term homeotic often is defined in the context of determinate animal development, i.e., transformation of tissues of one segment into those of another. But as Young (1983) and Poethig (1985) have pointed out, homeotic mutants occur in plants, although they usually have been described as teratological abnormalities. Bateson (1894, 1909) introduced the term homeosis (sic) to distinguish genetic changes per se from specific changes that result in "the transformation of a part into the likeness of another with which it stands in a series."

Several considerations make the pea leaf an attractive organ for the study of developmental regulation. The leaf is a determinate structure whose initiation is marked by a swelling (leaf buttress) on the flank of the apical dome (Lyndon, 1977; Meienheimer et al., 1983). A finite number of cell divisions, followed by cell expansion leads to the final mature form, a relatively uncomplicated structure comprising few cell types (dermal, vascular and ground tissue) and few cell layers.

The wild-type pea plant, i.e., the evolved, normal adapted form, is the standard to which all variation is related. Its leaves are compound and odd pinnate, being dissected into three morphologically distinct parts: two large foliaceous stipules; one or more pairs of leaflets (pinnae); and terminal tendrils (Fig. 1). Thus, the archetypal form is already segmented and specialized.

Repeatable and uniform leaf patterns occur not only between plants but also within the plant. Although the leaf itself is determinate, the stem axis...
on which the leaves are sequentially arrayed is indeterminate. The total number of leaves borne on a plant is governed chiefly by specific combinations of genes that control flowering behavior (Murfin, 1985). Leaves are produced throughout ontogeny, even after the onset of the reproductive phase. Whereas all the true leaves produced by the plant have similar morphological makeup (stipules, leaflets and tendrils), they become amplified during ontogeny. The first few true leaves (above the two modified leaves or cataphylls) typically have one pair of leaflets and a single terminal tendril. During ontogeny the number of leaflet and tendril pairs increases, reaching a combined maximum of about six or seven pairs (plus a terminal appendage). The plant's monopodial habit facilitates following and interpreting developmental events. Each node on the main axis contains several vegetative buds that ordinarily remain latent, but one or more of these may become activated in response to natural or experimental stimuli. This feature too offers certain advantages for the study of developmental processes. These and other favorable attributes of Pisum have led to a substantial genetic, physiological and biochemical literature.

Many mutants of pea are known to affect leaf size and shape (Blixt, 1972, 1974), but not all are convenient for analyzing their individual and combined effects in segregating populations (e.g., mutants controlling the length/width ratio of the leaflets). However, the mutants considered here exert distinct, unambiguous effects under a wide range of environmental conditions, and the phenotype peculiar to each mutant or mutant combination is manifest throughout plant ontogeny, despite the characteristic quantitative increase in appendage number (amplification). Individually the mutants act more to modify than to disrupt the wild-type leaf pattern. Most of the mutants are of spontaneous origin, most are long-established, and all but one are recessive. With one exception, all have been mapped to sites within specific chromosomes, and although some are linked, none of the linkages is extremely tight. Two mutants, tac and uni, appear to be allelic (Marx, 1986); plants with the uni allele exhibit pistilloid and complete sterility. All other mutants treated here are themselves fertile and give fertile offspring in crosses with wild-type plants and other fertile mutants. The described heritable variation thus lends itself to Mendelian analysis. Mutants within this group have been discussed principally in relation to phylogeny (Blixt, 1967; Gottschalk, 1970; Sharma, 1981) rather than to development.

The numerous phenotypes resulting from combinations of two or more mutants reveal aspects of gene action that are not evident in single substitutions, and it is these combinations that may elicit conceptual enlightenment regarding the regulation of leaf development in Pisum or of development in general. Mutant effects can best be seen and characterized in populations homozygous for one or more mutants but segregating for one or more other mutants. In the course of constructing such populations a series of isogenic lines has been created (Marx, unpublished).

The individual and combined effects of the following mutants will be reviewed: il, st, af, apu, tac, uni, sil, cob, cri, sel, ub, Td and half, the last two receiving minimal attention.

Individual Effects

The single mutants and their individual effects are briefly described below and all but one are depicted in Fig. 1, together with the wild-type phenotype from which they differ.

- **il**  (classica or tendril-less) (Vilmorin and Bateson, 1912). Chr. 7 (Lamprech, 1948). Laminar leaflets occur where tendrils normally occur, creating the acacia phenotype. Td is incompletely dominant in most genetic backgrounds, the heterozygous plants being distinguished by their strap-like tendrils (Fig. 2b).
- **st**  (stipules reduced) (Pellwe and Sverdrup, 1923). Chr. 3 (Lamprecht 1948). Causes a marked reduction in stipule size.
- **af**  (afida) (Kujala, 1953; Goldenberg, 1965). Chr. 1 (Khagildin, 1966). Tendrils occur where leaflets normally occur; lateral appendages highly branched, conferring a three-dimensional configuration in contrast to the quasi-two dimensionality of the normal leaf.
- **apu**  (apulvinar) (Harvey, 1979). Chr. 3 (Marx, 1984). Whereas leaflets of normal plants are mounted closely (nearly sessile) to the leaf rachis, the leaflets in apu (apulvinar) plants are borne on stalks or petiolules.
- **tac**  (tendrilled acacia) (Sharma, 1972). Chr. 3 (Marx, 1984). Leaves of tac (tendrilled acacia) mutants, like those of acacia (il) plants, terminate with a fully developed, laminar leaflet. Unlike il, however, tac plants have subterminal tendrils. Expression varies somewhat within and between plants. The lowermost true leaf often is unifoliolate; several subsequent leaves may be trifoliate. The typical expression of a single pair of tendrils usually occurs on leaves above node 5 or 6. Occasionally only one subterminal tendril is formed on a given leaf and more rarely two pairs of subterminal tendrils appear. The combined total number of lateral appendages appears not to exceed four.
- **uni**  (unifoliata) (Lamprecht, 1933). Chr. 3 (Lamprecht, 1948). Leaves of uni plants characteristically have a single leaf blade, in addition to normal stipules. Recently tac was found to be an allele of uni (Marx, 1986), and like tac, uni plants display some variability in expression. Certain nodes
may bear pinnatifid, bi-, or tri-foliate leaves, but the number of lateral appendages never exceeds two. Although tac and uni plants are readily distinguished, they often bear some leaves of similar morphology, viz. unifoliate or trifoliate leaves. Invariably, however, uni plants are completely sterile, whereas tac plants are fertile. Heterozygotes, i.e., tac/uni, usually can be identified because they are typically semi-sterile.

- sil (sinuate leaf) (Marx, 1977). Chr. unknown. Stipule and leaflet margins undulate or sinuate; terminal leaflets in sil tl plants also sinuate (Fig. 12).
- cob (cobbleata) (Wellensiek, 1959). Chr. 7 (Wellensiek, 1962). Stipules are absent from the first 5-6 nodes on cob plants. Above this, stipules may be narrow, sessile and elliptical, spoon-shaped (the most common expression, Fig. 1), thread-like, strap-like or they may be transformed into a structure resembling normal leaves, with fully organized leaflets and tendrils (Fig. 7). Some plants may exhibit several or all of these variations.
- cri (crispa) (Lamm, 1949). Chr. 5 (Lamm, 1949). Stipules and leaflets are distorted and revolute. Veins are thick and prominent. The first pair of leaflets is inserted close to the stem axis, i.e., the petiole is markedly shortened in comparison with non-mutant plants (Fig. 1 and 9). Moreover, the petiole characteristically is winged and the stipules often are fused with the wings and with the first pair of leaflets. Pleiotropic effects on the reproductive apparatus result in reduced seed set, but the plants have normal male and female fertility.

Some mutants do not affect leaf architecture as such but nevertheless do cast light on developmental events. Among them are mutants that control epicuticular wax formation and distribution. Whereas all above-ground parts of wild-type plants have a thin wax coating, certain mutants reduce or eliminate wax on non-mutant leaves. Two such mutants are considered here:

- uko (wachslos or supra-inserata) (Nilsson, 1933). Chr. 6 (Lamprecht, 1948). Upper surfaces of leaflets with little or no wax, all other surfaces are normal waxy.
- uco (vix-conerta) (Vilmorin, 1913). Chr. 2 (Lamprecht, 1948). All leaf parts except the upper surfaces of leaflets with little or no wax. In effect, therefore, the phenotype of uco is the photographic negative of uko. Waxless surfaces are shiny whereas waxy surfaces are dull.

Two examples of mutants that affect leaf margins and therefore may be late-acting (or early-acting?) mutants will be described but not discussed further. They are also among those that affect all component parts of the leaf.
Fig. 4. Leaves (with stipules removed) each with a common genotype except for tac (right). Reduced branching and larger leaflets mark presence of tac.

Fig. 5. Leaf illustrating additive effects of apu, tac and eeb.

Fig. 6. Stipules with adventitious tendrils arising from clefts in stipule tips resulting from interaction of af and sil.

-Tld (Scalaris forma) (Wellensiek, 1925). Chr. 4 (Lamprecht, 1948). Stipule and leaflet margins are dentate on leaves formed above node 5 or 6. Dominance in this, the only dominant mutant in the group under discussion, is incomplete.

-bulfl (burnt leaf) (Sharma, 1973). Chr. 3 (Marx, 1980). Leaf margins become necrotic, brown and papery. Significantly, not only are stipule and leaflet margins necrotic, but so too are the tips of the tendrils (Fig. 19).

Considered individually, i.e., as single allelic substitutions into an otherwise normal genetic background, the various leaf mutants appear to operate in different domains or compartments of the leaf. If a given mutant's pheno-

typic effect defines the boundaries of the compartments as is the case in the homeotic mutants of Drosophila (García-Bellido et al., 1973, 1979), then st, af, and tl define three compartments: stipule, leaflets, and tendrils, situated in the basal (proximal), middle, and terminal (distal) portions, respectively. That the terminal compartment itself may be composed of subcompartments is suggested by the mutant tac (terminal leaflet with subterminal tendrils). Three of the mutants listed, tl, af, and tac, fit the definition of homeotic because various leaf parts become transformed to resemble parts normally located elsewhere in the leaf. This conclusion is reinforced by the demonstration of Gould et al. (1986) that the tendrils of the af leaf are true tendrils,
one mutant masks the effect of another (at least at the gross morphological level). Evidence of pleiotropy was not observed. Generally only the gene symbols representing the mutant form are given; all other loci can be assumed to be wild-type (non-mutant). Occasionally, however, the symbol for the dominant non-mutant allele is given when it seems to add clarity.

**Additive Effects**

Least informative is the additive effect wherein each mutant apparently acts independently of the other and the phenotype represents the sum of the two (or more) effects, e.g., \( st + tl \) (Fig. 3b), \( st + af \) (Fig. 3a), \( st + tac \), \( apu + tac \), \( apu + tac + cob \) (Fig. 5). Such additive effects, however, may argue that the pea leaf is indeed organized into compartments. Although not strictly additive, some mutant combinations help define the limits of action of certain other mutants. For example, in a wild-type background the effects of \( wlb \) and \( apu \) are manifest only in the middle segment of the leaf, whereas leaflets occur. Neither affects the stipules and their effects cannot be discerned in the terminal portion of the leaf occupied by tendrils. By introducing \( tl \) and thereby converting tendrils into leaflets, the influence of \( wlb \) and \( apu \) can be seen to include the distal part of the leaf as well. But \( tl \) (or \( tac \)) is required to demonstrate this. Similarly, \( wb \) and \( sil \) expresses in both the basal (stipules) and middle portions of the leaf; their influence on the distal third cannot be detected as long as it is occupied by tendrils. With the introduction of \( tl \), i.e., \( wb \) \( tl \) and \( sil \) \( tl \), the terminal leaflets are waxless on the abaxial surface in the case of \( wb \) and sinuate in the case of \( sil \) (Fig. 12), demonstrating that both \( wb \) and \( sil \) influence all three parts of the leaf.

**Epistatic Effects**

Combinations that reveal epistatic action give insight into the relative strength of one developmental impulse compared with that of another. Epistasis may be as powerful as the dominance relationship to help apprehend the overall developmental process. For example, in the combination \( tl + uni \), the presence of \( tl \) cannot be visually detected because both \( uni \) alone and \( uni \) \( tl \) are unifoliate. Similarly, \( af \) conceals the presence of \( apu \) (unless additional mutants are also present). Slightly more complicated is the \( tl + tac \) combination (Fig. 2 a,c). Here \( tac \) exerts an epistatic effect over \( tl \) by limiting the number of lateral leaflets on a given leaf (Marx, 1986) and by increasing the surface area of the terminal leaflet (Sharma, 1981). In the very same combination, however, subterminal tendrils are absent, so in this respect \( tl \) is epistatic to \( tac \) (Marx, 1986). Dominance relations also come into play in this combination. In lines homozygous for \( tl \) (\( tl/tl \)) but segregating for \( tac \) (\( Ta/tac \)), the
Fig. 12. Plant showing combined effects of tl and sil. Note that leaflets borne at the terminal portion of leaf as well as the stipules and middle leaflets are sinuate.

Fig. 13. Mutant sil in af tl background. Note adventitious tendrils emerging from stipule rips, the phenotype of which mimics the configuration (pleiofila) of the leaf as a whole.

Fig. 14. Stipule with waxless tip from a sil solo plant. In plants with solo alone the entire stipule surface has a wax coating.

Fig. 15. Stipule with a distinctive outgrowth resulting from an interaction of cri and sil.

Fig. 16. Phenotype of plant with af tac combination. Note small leaflets scattered amid clusters of tendrils.

Fig. 17. Left: Seedling with genotype af St apu tac tl. Right: af St apu tac tl. Apu-apu allelic difference can be detected in these plants; whereas apu is obscured in plants with af alone.

Fig. 18. Leaf with combined expression of tac and solo. Upper surfaces of leaflets, including terminal leaflet, are waxless (shiny).

Fig. 19. Plant bearing the single mutant, balf. Tips of tendrils as well as margins of stipules and leaflets are necrotic, brown and papery.
plants with fewer leaflet pairs identify the tacltacl segregants. In lines homozygous for tac (tacltacl) but segregating for tl (Tl/tl), the tac tl plants are as described, but the Tl/tl tacltacl plants can be discerned from the two homozygous classes (Tl/Tl tacltacl, Tl/tl tacltacl) by the strap-like subterminal tendrils (Fig. 2c). This observation suggests that the subterminal tendrils produced by tac plants are anatomically similar to the tendrils of normal plants, just as are the tendrils of af plants (Gould et al., 1986). Since tac and uni have some phenotypic similarities (both limit the degree of structural ramification, uni more than tac), and since the two mutants are allelic, the restricted number of leaflet pairs in tac tl and in uni tl appears to be a quantitative reflection of the difference between tac and uni, with uni exhibiting the stronger effect. This reduction applies also to the af tac (Fig. 4) and af uni (Fig. 11) combinations, both having fewer secondary and tertiary branches than af Tac plants.

Interactions
Interactions yield novel phenotypes, i.e., unlike any of the individual mutants participating in the interaction. No one mutant masks the effect of another, but the contribution of each mutant is evident in the phenotype. The interaction of af and tl is a notable example (Figs. 5c, 13): the production of numerous, small laminae structures (pleiofila) is characteristic of neither mutant acting singly. However, the highly ramified nature of af is retained in af tl plants, and, although laminae are smaller and far more numerous in af tl than in tl alone, laminae are present where none exist in af alone. Thus, features of both mutants are expressed in the double recessive plants. Viewed from the standpoint of compartments, the middle and terminal compartments are no longer identifiable as distinct parts. Instead, they appear to form a single, undivided spherical array of minute leaflets. This view is strengthened by the fact that in af plants heterozygous for Tl (i.e., af Tl/tl) all the tendrils on the entire leaf are strap-like, not just the tendrils at the distal portion of the leaf. Stipules remain unaffected.

Alone, sil produces sinuate or undulate stipules and leaflets. Apart from the fact that sil affects all parts of the leaf, as demonstrated by the fact that the terminal leaflets of sil sil plants are also sinuate (Fig. 12), the mutant phenotype appears otherwise unexceptional. It is in combination with certain other mutants that sil exerts its singular and profound effects (Marx, 1977). In an otherwise wild-type background, the wax mutant, uwo, reduces or inhibits wax formation exclusively on the upper surface of the leaflets; the lower leaflet surfaces, the rachis and both upper and lower surfaces of the stipules remain waxy. When sil and uwo are combined in the same plant, however, the stipule tips, along with the upper surface of the leaflets, become waxless (Fig. 14). In contrast, when sil is combined with wb the stipule tips, which without sil are waxless, now become waxy. Hence, in the case of uwo sil, mutant tissue appears at a location where it does not usually appear, whereas in the case of wb sil, non-mutant tissue appears where, without sil, mutant tissue ordinarily appears.

In an af background, sil elicits an even more dramatic phenotypic effect (Marx, 1977). Plants with this combination produce stipules with deeply incised stipule tips from which issue adventitious tendrils (Fig. 6). The adventitious tendrils resemble ordinary tendrils if the plant is dominant at the Tl locus. However, in plants with the combination of af sil, the adventitious emergences mimic the phenotype generated by the af tl combination (i.e., the pleiofila phenotype) (Fig. 13). Moreover, in plants heterozygous for Tl (af sil Tl/tl) the adventitious tendrils assume the strap-like character typical of tendrils borne on leaves of Tl/tl plants. Although the stipules of nearly all leaves borne on af sil plants become strongly distorted or convoluted, not all become incised and bear adventitious tendrils.

However one interprets the striking phenotypic alterations associated with the presence of sil, they constitute transformations of a homeotic nature. In this case, however, the resulting phenotypes are not attributable to the effect of a single mutant acting in a wild-type background, but rather to the effects of unlinked mutants interacting among themselves. Overall, the observations may suggest evidence of homology between the stipule base and the abaxial surface of the leaflet on the one hand and the stipule tip and the adaxial leaflet surface on the other (Marx, 1977).

Just as the full implications of sil are revealed only through interactions with other mutants, so too do gene interactions reveal additional insights into the action of af.

Combining tac and af yields a phenotype with small laminae scattered amid a cluster of tendrils (Fig. 16). Thus, whereas af in combination with tl converts the entire leaf structure distal to the stipule into tiny laminae, the combination af tac more nearly resembles the phenotype of af alone except that the cluster of tendrils includes several small laminae (but these are decidedly larger than in the af tl combination). Moreover, af tac plants heterozygous for Tl (Tl/tl) resemble the phenotype of af tac Tl/tl except that all the tendrils are strap-like. This implies that only the leaflets borne in the second or middle segment of tac plants are transformed into tendrils upon incorporation of af. In the presence of af, the influence of tac appears to be confined to the extreme distal portion of the leaf, whereas, the Tl/tl locus affects both the distal and middle leaf parts. Overall, the af tac combination has additive properties in that the middle leaf segment is composed predominately of
tendrils; it has epistatic properties in that the leaf is less ramified than in the 
af leaf alone; and it is interactive in that more than one lamina is produced 
per leaf, some of which are borne laterally and all of which are smaller than 
the terminal leaflet present in plants carrying tac alone, but larger than in af 
tl plants.

The entire leaf structure (above the stipules) is composed of small laminae 
in the triple combination af tac tl, but the surface area of the individual 
laminae is significantly greater in such plants than in af tl (Fig. 4). This 
difference in leaflet size relates to a difference in expression between tac and tl 
in a normal (Af) background (see previous section). But now, all leaflets 
comprising the cluster in af tac tl plants are larger than in af tl plants, not merely 
the distal leaflets. Thus, the presence of af tl may allow tac to influence the 
morphology of the middle portion of the leaf; whereas it seems unable to do 
so in af tac Tl plants.

The presence of laminar tissue on an otherwise tendrilled plant (af tac) 
revealed that af inhibits the expression of the wax gene, wlo (Marx, 1984). 
Evidence for this is indirect and, again, is made manifest through mutant 
interactions. The usual expression of wlo is not altered in Af wlo tac plants, 
the upper surfaces of the leaflets — including the terminal leaflet — being 
watery while both surfaces of the stipule and lower surface of the leaflets 
remain normal waxy (Fig. 18). Since af wlo Tac plants have no leaflets, the 
presence of wlo cannot be detected, suggesting that wlo expression is merely 
concealed for lack of laminae on which to become expressed. But because af 
wlo tac plants (unlike af wlo Tac) produce some laminar tissue, it is reasonable 
to expect that the upper surface of such leaflets be waxless. They are instead 
waxy, as is evident both from visual observations and from SEM micrographs 
(Marx, 1986, unpublished). Apparently, therefore, af not only obscures wlo 
expression but actually inhibits it. These observations prompted examination 
of plants of the phenotype af wlo il (pleiofila). Here too wax was present on the 
upper surface of the laminae, further supporting the belief that af counteracts 
the effect of wlo. The epistatic effect of af on wlo is shown in still 
another, still more subtle and indirect, way. Stipule tips of af wlo sil tl, as 
already mentioned, develop clefs from which arise adventitious tendrils. The 
laminae of such emergences produce wax on both adaxial and abaxial surfaces. 
Furthermore, although the stipules of af wlo sil tl or af wlo sil Tl plants 
become incised and thereby obscure the status of wax deposition of the tips 
of those stipules, stipules on the lower leaves do not become incised. The 
stipule tips on the lower leaves should be waxless in af wlo sil plants unless af 
inhibited wax deposition. Such stipule tips are waxy. Although wlo inhibits 
wax formation and af restores wax formation, it is not known if the pheno-
typic differences reflect a change in wax biosynthesis (i.e., a chemical switch), 
or if this phenotypic difference is a product of morphological alteration. However, 
the presence of wax on the stipule tips of af wlo sil favors a biochemical 
explanation.

Combining af and uni results in another distinctive phenotype (Fig. 11). 
The leaves show little amplification, thereby reflecting the influence of uni. 
Many leaves bear a striking resemblance to those produced in tac plants, i.e., 
a terminal lamina leaflet with a pair of subterminal tendrils. Although the total 
appendage number is low, a relatively high proportion of the leaf is composed 
of laminar tissue, the blades being larger than in af tac plants. Leaves formed 
late in ontogeny may have two or three blades tightly clustered at the leaf apex. 
With respect to the expression of wlo, as discussed in the above paragraph, 
the upper surface of laminae on Af wlo uni are waxy, but they are waxy in 
af wlo uni plants, just as they are in af wlo tac. Since the laminae are larger in 
af wlo uni than in af wlo tac, the inhibitory effect of af on wlo is especially evident.

In af apu plants, the presence of apu cannot be detected. However, when 
tl, tac or uni are present along with af and apu, then the presence of apu is 
again manifest (Fig. 4, 17). In af apu plants the petiole bearing the single 
blade is noticeably elongated (Fig. 10); whereas in other gene backgrounds 
apu increases the length of the petiolules only.

The phenotype of the cob cri combination reveals two features not found in 
plants possessing either mutant alone. Whereas the petiole length is significa-
cantly foreshortened in plants with cri alone, in cri cob plants the petiole 
length is not equal but the petiole length of normal plants (Blixt, 1967). 
Secondly, stipules are virtually absent over the entire length of the stem 
axis in cri cob plants; only a thread-like vestige of the stipules remains 
(Blixt, 1967). The stipules of cri st plants also are reduced to diminutive 
spines which are considerably smaller than those found on st plants alone (Fig. 
9). Evidently cri has a fundamental influence on stipule morphology as well 
as on the rest of the leaf. Thus, three combinations, viz., cob cri, cob st and 
cob cri st result in stipules that are either absent or sharply reduced.

A characteristic of cob plants which perhaps transcends all others is the 
replacement, at least at some nodes, of stipules with leaf-like structures and, 
in some instances, the leaf-like stipules mimic the phenotype of the "true" 
leaf (Gottschalk, 1972; Sharma, 1981). The leaf-like structures that match 
the phenotype of the true leaf appear most consistently in cob lines carrying 
af (Fig. 8).

The morphologies resulting from most mutant combinations exhibit little 
if any loss of tissue integrity. Some, however, particularly those involving cri
together with af, sil, tl and tac, generate bizarre phenotypes wherein certain tissues appear somewhat disorganized. In such cases tissues may vacillate between tendril-like, blade-like or intermediate (funnel-shaped) structures. Stable novel phenotypes have also been observed, for example that associated with the combination cri sil. Recall that in af sil plants tendril-like structures arise from deeply incised stipule tips, but the stipule tips are not incised in Af sil plants. In Af cri sil plants, a distinct outgrowth arises from the stipule tips but the outgrowth appears to be continuous with the tissue at the stipule apex (Fig. 15). Thus cri interacts with sil in a manner similar to but not identical with af. Combinations involving cri are especially effective in revealing a rather high degree of continuity between the stipules and distal parts of the leaf.

Discussion

All but a few studies of the histogenesis, anatomy, morphology and ultrastructure of Pisum have involved the normal (wild-type) leaf form (Reeve, 1948; Mitra, 1950; Sachs, 1969; 1977; Lyndon, 1977; Meichenheimer and Muehlbauer, 1982; Hussey, 1972; Hussey and Gunn, 1984). Only recently have mutants become the desideratum. Meichenheimer et al. (1983) examined the developing meristems of lines isogenic for normal, af, tl, and st as well as the double and triple recessive combinations. Likewise, Gould et al. (1986) used isogenic lines to describe and compare development of a normal leaf with leaves of af and of tl; they also devised an in vitro culture system for axillary shoots to facilitate studies of leaf morphogenesis. Both studies showed that differentiation occurs early, and that development proceeds acropetally. Although the material investigated and the experimental findings were generally similar in both studies, there were distinct differences in emphasis and interpretation.

The experimental observations of Gould et al. (1986) were interpreted in relation to an algebraic model for compound leaf development advanced by Young (1983). One essential assumption in Young’s model is that the developmental fate of a meristematic primordium depends on its size at a critical stage. It requires no information about its position or its previous developmental history (Young, 1983). A primordium will become a rachis, leaflet or tendril depending on descending order of its size, and an alteration in one leaf part need not affect any other since the meristems are postulated to be independent. Young accounted for the effects of af and tl by assuming the mutants alter the size threshold values. Thus, normal leaf form in Pisum is reckoned in terms of a sequential ordering of primordium size and that departure from the norm resulting from the action of mutant genes are related to thresholds and responses to primordium size.

Meichenheimer et al. (1983) stressed the various types of meristems that come into play during the ontogeny of normal and mutant leaves. Morphological differences were explained on the basis of whether or not or for how long various defined meristems are active. Thus, the genetic control is exercised via differential activation, repression (inactivation) or duration of primordia in the leaf, resulting in modifications of morphology. These authors, too, have recognized the apparent independent genetic control over the formation of leaflet, stipule and marginal meristems. In the af leaf, marginal meristems are completely absent, whereas in st, there is an early loss of stipular marginal meristems. They therefore view af as responsible for eliminating an essential component in the ontogenic meristem; whereas st affects the duration of marginal meristems function. In the case of tl, the mutant gives rise to marginal meristems where none exist in the normal leaf. Although not expressly stated, the authors seem to consider tl as controlling the fate of the terminal appendage exclusively rather than the terminal portion of the mature leaf.

Neither Meichenheimer et al. (1983) nor Gould et al. (1986) studied the phenotype produced by heterozygosity at Tt (Tt/tt) wherein the tendrils are modified into strap-like structures. Presumably, the incomplete dominance of Tt reflects an influence on the duration of marginal meristem activity. In any event, because one to three pairs of subterminal tendrils of Tt/tt plants may become strap-like, this clearly indicates that the entire distal portion of the leaf is under the influence of Tt. Since the total number of lateral appendages per leaf increases during ontogeny, the number of lateral appendages comprising the distal portion changes correspondingly. Recognition of this affects the interpretation of the actions and interactions of all the mutants under consideration.

Stipules have been interpreted variously, as independent organs at one extreme or as an integral part of the leaf at the other (Eames, 1961; Rutishauser and Sartel, 1986). The lack of agreement appears to reflect worker proclivities and the wide differences in anatomy and morphology found among the diverse taxa studied. Although Mitra (1950) considered the stipules of peas to be part of the leaf, nevertheless he stated that “the stipules and leaflets belong to different categories.” Unlike the leaflets, the stipules of peas have no separate primordia but instead are direct upward divergences of the lateral arms of the base (Mitra, 1950). The vascular bundles that serve the stipules are not the same as those that serve the rest of the leaf (Sachs, 1977). The
genetic evidence offered here appears strongly to favor the view that the stipules in peas are an integral part of the leaf.

The homeotic mutants of *Drosophila* were key in the formulation of the concept of compartmentalization, wherein development is characterized by a series of compartments and subcompartments formed by a stepwise process under the control of genes that select the appropriate developmental pathway. Although this idea has gained wide acceptance, Wilkins (1986) discusses aspects of the concept for which there is room for debate. That the pea leaf is composed of compartments is suggested by the observations presented. Whether or not the pea mutants that qualify as homeotic are any more relevant than the other genes in the system in establishing the existence of compartments or indeed of shedding light on leaf morphogenesis only future investigation can determine. Recently, Christianson (1986) used chimera clonal analysis (fate mapping) of the developing embryo to adduce evidence of compartmentalization in the cotyledons of cotton seedlings. The pea material would be well suited to clonal analysis using one or another of traditional methods of inducing chimeras.

Various studies are consistent in showing that the pea leaf becomes determined and differentiated at a very early stage in development. Surgical manipulations performed in the buttress stage indicate that the leaf is capable of regeneration at that stage but rapidly and progressively becomes restricted developmentally (Sachs, 1969). The transition from the undeveloped state occurs in a span of about one plastochron (or one node or 1-2 days). At the time a leaf primordium of a growing plant becomes determined, all the leaves borne on the preceding nodes are of course already determined and differentiated and the lowermost leaves may be fully mature or senescent.

Since all the leaves formed before and after the last-developed leaf faithfully repeat the same leaf pattern (at least qualitatively), the same "message," be it the wild-type or mutant message, is transmitted acropetally along the developing axis. Moreover, the same instructions are sequestered in the latent buds present at each of the successive nodes on the main axis. When activated, these buds give rise to secondary branches whose leaves exhibit the same leaf phenotype as those arising from the main stem. Tertiary branches arising from secondary axes behave similarly. So rapidly do the activated axillary buds become organized shoots that if they are not already fully determined, they almost certainly are competent. But this point can be extended further. The embryo of a mature pea seed contains 5-6 nodes which are already differentiated. It therefore may be of some value to examine over time the developing seed (borne on the maternal parent) of different genotypes in search of critical biochemical (or anatomical) differences that may be associated with differences in leaf pattern.

Considering the question: Is there a developmental program? Wilkins (1986) draws attention to important distinctions between a putative "genome program" (for development) and a "gene program" (for polypeptide synthesis). The former, unlike the latter, requires utilization of information selectively drawn from throughout the genome, and the information so selected at any given step in the process indirectly determines what information will be utilized at the next step. He points out that information of this kind is not presently known to exist in the genome and underscores the point with the example that hybrids of certain reciprocal crosses, despite an identical genotype, have different phenotypes because their cytoplasms are different. These realities pose formidable challenges to the developmental biologist.

Wilkins (1986) offers a brief critique of the strengths and limitations of several proposals to account for the regulation of pattern formation in animals. Among these, Wolpert's (1971) positional information hypothesis is cited as the most widely accepted. Poethig (1984), however, in reviewing what little is known about the control of leaf morphogenesis in angiosperms, concludes that the Wolpert model cannot be applied to plants. Whether the arguments Poethig invokes to support his views are sufficient to rule out in part or in total the animal model(s) remains to be seen. For that matter, whether any existing model of pattern formation will prove satisfactory, is an open question. Progress toward an understanding of pattern formation in plants is not likely unless or until considerably more research is conducted.

**Conclusions**

Numerous authors have cited the dearth of general principles of development upon which to test the plethora of observations. Whereas this review scarcely mitigates that shortcoming, perhaps the observations described here, if experimentally pursued, may lead to some fresh insights. This hope is prompted by certain features of the system, any or all of which may confer specific advantages, whatever the approach or level of attack.

- A succession of essentially reiterated determinate structures borne on an indeterminate axis; the determinate structures are subject to extensive monogenetically controlled variation with large phenotypic differences; and the indeterminate axis likewise is subject to genetic and environmental manipulations (thus the number of leaves on a given plant can be experimentally controlled).
- Cell division presumably plays a predominant role and cell differentiation
and cell migration play a subordinate role, if any at all. Although this may be a simplifying feature, it is recognized that regulation of pattern formation is perhaps the most refractory aspect of development.

- Although the successive leaves in a given plant become amplified during ontogeny, they do not become materially more complex, differences in pheno-
typic expression being primarily a reflection of differences in spatial arrangement. The mutants discussed operate to modify rather than fundamentally change normal leaf architecture.

- The availability of isogenic lines and of lines in which certain mutants segregate with a background of one or more other mutants represents a clear aid to any investigation, regardless of its nature.

The present system of mutants is seen as a potential adjunct to support a range of different disciplinary approaches to the question of developmental regulation. Several studies cited earlier attest to the useful role mutants can play.

Many of the leaf morphotypes generated by the mutants within this sys-
tem, when compared with the phenotypes of allied taxa, show similarities and differences which often are large and qualitative in nature (i.e., of the kind described here). These morphological differences, whether they reflect analogy or homology, may possibly reflect common control mechanisms. There may be evolutionary implications as well.

References

A Suite of Mutants

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