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Independent responses of two fruit characters to developmental regulation in *Microseris douglasii* (Asteraceae, Lactuceae)[1]

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Summary. The 'hairy achenes' and 'yellow achenes' characters are expressed only in peripheral fruits on *Microseris* capitula. Segregation in interstrain hybrid D37 shows that the genes responsible for these characters respond independently to developmental regulation.
Key words. Microseris douglasii, fruit, peripheral; achenes phenotype; genetic analysis; morphogen gradient.

The detailed course of the development of leaves, flowers and other organs in herbaceous plants is usually dependent on the position of the developing organ with respect to the apical meristem or, more exactly, on the position of the organ primordium in the sequence of primordia elaborated by the meristem. If there are marked morphological changes in sequentially formed organs, the effect is termed 'heterophylly' for leaves or 'heterocarpy' for fruits. Bachmann[2] has discussed various manifestations of this effect and suggested that it can be understood as the response of the developing primordium to concentration differences of a morphogen (phytohormone?) that depend on the diffusion gradient of that morphogen and/or the time course of its production. Even where this system is not amenable to direct physiological experimentation, it can be analyzed in considerable detail by genetic dissection of its components. Specifically, 2 types of genes should be found; genes that participate in the establishment of the morphogen gradient and genes that are expressed under the influence of the gradient.

The flowering head (capitulum) of the Compositae (Asteraceae) is a particularly suitable system for such a genetic analysis. The development of each of the many individual florets and of the fruits (achenes) that mature from them can be considered a bioassay for the morphogen gradient that can be read in the mature fruiting head. With tens or even hundreds of achenes present on a single capitulum, the gradient can be probed very precisely. We have used a specific fruit character, the 'hairy' fruit wall of the outer, older achenes in contrast to the smoother wall of the inner ones, to probe the genetic basis of heterocarpy in the annual species of *Microseris* (Asteraceae: Lactuceae)[3,4].

At least 2 genes interact in the analyzed strains to determine the relative number of hairy achenes. These appear to be genes determining the gradient[2]. Their interaction suggests that the most effective concentration (the origin of the gradient?) is around the rim of the capitulum. From there centripetally a more or less broad ring of hairy achenes is found that encircles an inner field of smooth ones. The existence of 'half-hairy' achenes at the border between outer and inner ones suggests that the response to the gradient is cell-specific and can vary across a single organ primordium[2].

The 'hairy achenes' phenotype is one of many characters under the influence of this gradient. These include the shape, color and color pattern of the flower petals, the shape, color, and color pattern of the fruits and the structure of the pappus. The analysis of these other characters is very difficult, because the flower characters must be scored during the short period of flowering, most fruit characters cannot be scored in sterile aborted fruits that usually occur in most capitula, and the pappus characters show a remarkable environmental plasticity obscuring a complex genetic basis. The analysis of a coordination (or a lack of it) among the responses of different genetically determined characters to the morphogenetic gradient across the capitulum is therefore limited to exceptional favorable cases. Such a case is presented here. We shall demonstrate segregation for the dependence of fruit color on the position of

the fruit on the capitulum in the absence of segregation for the 'hairy achenes' character. In terms of our gradient model this would correspond to segregation for the response threshold of the color alleles to a constant gradient.

We have found this in the F2 generation of the interstrain hybrid D37 of *Microseris douglasii*. This is a family of 30 plants obtained by spontaneous selfing from an F1 hybrid specimen between the inbred strain D40 (derived from a population at Cholame, San Luis Obispo County, California) and inbred strain B14 (derived from a population at Parkfield, Monterey County, California) as pollen donor. The following properties of these plants are a rare favorable constellation of circumstances for a straight-forward analysis: Both parental strains show the 'hairy achenes' character in precisely the same proportion of their achenes. The expected mean frequency of hairy achenes in both cases is 11.47%. Due to the geometric arrangement of the floret primordia, the proportion of one type of floret or fruit in the total number corresponds roughly to the ratio of 2 numbers in the Fibonacci series (1,1,2,3,5,8,13 .....) which converges between subsequent members of the series to the value 0.618.... and between more distant members to integral powers of this ratio. We therefore use the logarithm base 0.618 (the 'lf value') of such ratios in order to obtain simple numerical values with additive inheritance[3]. The lf value for the proportion of hairy achenes in both parental strains is limited between 4 (14.59%) and 5 (9.02%) with a mean at 4.5 (11.47%), (fig.). All hairy achenes have a brownish yellow ground coloration which contrasts with the dark brown ground color of the inner achenes.

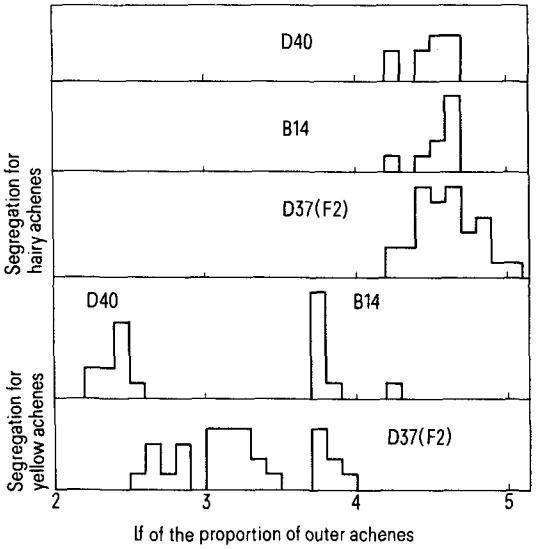
The 'yellow achenes' character in both strains extends centripetally beyond the hairy achenes so that a ring of smooth yellow achenes is situated between the outer ring of hairy yellow and the inner disc of smooth dark brown achenes. The transition between the yellow and the dark brown coloration is exceptionally abrupt in both parental strains. Often such color differences fade more gradually into each other along the radius of the capitulum. While the proportion of hairy achenes is very closely the same in both parental strains, the zone of yellow achenes extends markedly farther towards the center of the capitulum in strain D40 than in strain B14 which has very few smooth yellow achenes. Since the number of yellow achenes in both cases includes all the hairies, their proportion is higher than that of the hairies, and the lf values for the proportion of yellows therefore are smaller than those for the proportion of hairies. The respective values are just below 4 (circa 15% yellows inlcuding hairies) for strain B14 and near 2.5 (circa 33%) for strain D40 (fig.). These different proportions are stably inherited in the parental plants and offspring derived by selfing from them and raised at the same time and under the same conditions as the hybrid plants.

The hybrid strain D37 derived from a D40 × B14 cross produces very vigorous and nearly completely fertile plants so that the possible error in the color score of the few aborted achenes can be neglected. All lf values are based on the totals from the first 5 mature heads of each plant (circa 400 achenes per specimen).

The figure shows that the F2 plants have essentially the same percentage of hairy achenes as both parental strains. The identical phenotypes of the parents therefore reflect an identical genetic basis. This contrasts with a clear and simple segregation for the proportion of yellow achenes. Many of the F2 plants have values intermediate between the parental ones. 7 out of 30 plants (23.3%) have the parental B14 phenotype. This indicates that there is a single pair of 'yellow achenes' alleles segregating. The plants with the D40 genotype have 1 or 2 fewer yellow achenes per head than expected. This slight shift to higher than expected lf values can also be seen in some of the scores for hairy achenes.

The most likely interpretation of these results in terms of our gradient model is the following: the genetic basis of the 'hairy achenes' phenotype is identical in both parental strains. This includes the determination of the same morphogen gradient in both strains. The single gene difference in the 'yellow achenes' phenotype therefore reflects differences in the response thresholds of the 2 'yellow achenes' alleles (or their controlling elements) to the morphogen gradient. Independent of the detailed genetic mechanism, these results are a clear and straight-forward demonstration for a very important aspect of the developmental genetics of plants: (at least) 2 genes involved in fruit development are independent in their (response to) developmental regulation. Regulation is at the level of the individual gene in the individual cell, and there is no evidence and no need for an organ-specific 'integrator gene' that mediates between the stimulus for organ induction and the set of genes involved in the response to that stimulus.

Such integrator genes are part of the Britten and Davidson model of gene regulation in differentiation[5]. The genetic variation found here may well concern *cis*-regulatory DNA sequences shared by all genes dependent on one specific morphogen. Such sequences play a role in every model of gene regulation including that of Britten and Davidson[6]. Relatively short repetitive sequences with some of the postulated properties of *cis*-regulatory elements have been identified in inducible genes of yeast[7], *Drosophila*[8], and the mouse[9].



Segregation for the proportions of hairy (above) and yellow (below) achenes in 2 inbred strains of *Microseris douglasii* (D40 and B14) and in the F2 of their hybrid (D37). Numbers on the abscissa are lf (logarithm base 0.618) of the proportions of each achene type.

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