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# The pattern of mutation of an unstable gene in *Delphinium ajacis*

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Among the recognized genes that control flower colour in *Delphinium ajacis* is one which, while normally allowing the accumulation of a pink pigment in the sepals, mutates at a high rate to an allele that enables a blue pigment to be formed. This unstable gene has been designated $p^*$ (Dawson, 1955). The extensive work of McClintock on unstable genes in maize (McClintock, 1948–1962) has revealed patterns of behaviour which can be interpreted in terms of transposable genetic elements which either induce mutation or suppress the expression of the gene to which they are attached or both. She has called these elements 'controlling elements'. Some of the examples that McClintock has described are two-element systems in which the activity of the controlling element depends on the presence of a second controlling element at some other location in the genome. A further feature of most of these systems is that the complex of gene and attached controlling element can 'change its state' and show a different pattern of instability from that shown previously. The aim of the present study was to see whether the pattern of instability of the $p^*$ allele in *Delphinium ajacis* was similar to those in maize that were due to controlling element systems. In the course of this work fairly extensive data were collected on the rate of mutation of the $p^*$ allele in somatic and germinal tissue in both homozygotes and heterozygotes and these will be discussed in more detail than other aspects of the pattern of the instability.

## 1. THE INHERITANCE OF DIFFERENCES IN FLOWER AND FOLIAGE COLOUR

In the stocks that I have studied the $p$ factor consists of four alleles (Dawson, 1955). $p$ is recessive to the others and produces evenly coloured pink flowers while $p^b$ is dominant and allows the formation of blue pigment. $p^l$ is the third stable allele; the genotype $p^lp$ has lavender flowers while $p^lp^l$ has dark lavender flowers that are often difficult to distinguish from those with the $p^b$ allele. The $p^*$ allele in a $p^*p$ plant mutates to $p^b$ to produce pink flowers that have numerous spots and sectors of cells containing blue pigment. These spots and sectors are surrounded by a line of cells which contain what appears to be a trace of blue pigment; arising presumably by diffusion into them of the blue pigment, or a precursor, from the mutant cells. The intensity of pigment does not increase in either the mutated or the halo cells after the flowers have fully opened, so the reactions involved in its production appear to cease at this stage of development. The presence of spots consisting of a single cell

with blue pigment demonstrates that a mutant cell does not have to divide before the mutant gene is expressed; there is no evidence of delayed phenotypic expression of the $p^b$ gene. The flowers of $p^*p$ have stable pink areas and sectors. $p^*p^*$ plants have flowers of a similar phenotype to those of $p^*p$ except that the density of blue spots and sectors is higher and stable pink sectors are much rarer. $p^*p^l$ plants are the same as $p^*p$ plants except that the background colour of the blue mutant areas is lavender instead of pink. Crosses between different $p$ genotypes have consistently shown good agreement with the expected mendelian ratios apart from a slight deficiency of effective $p^l$ female gametes, and a rather larger deficiency of effective $p^l$ male gametes, from heterozygous plants.

In one of numerous $p^lp^* \times pp$ crosses there was one plant which had lavender sepals with blue spots and sectors. It was possible that the $p^l$ allele had become unstable but on crossing this plant, as pollen parent, to $pp$ the offspring were 15 lavender (probably $p^lp$), 22 unstable pink (probably $p^*p$) and 23 pink (probably $pp$). The absence of any unstable lavender makes it unlikely that the $p^l$ allele had become unstable. It is more likely that the original plant was a trisomic ($pp^*p^l$) and only pollen without an extra chromosome effected fertilization, as in maize. Equal numbers of each class of offspring would then be expected and the results agree with this.

The only variant foliage colour was a pale yellow-green. Plants of this type are weak but grow to flowering and can be crossed. It can be scored in the seedling stage and is inherited as a recessive ($gg$). An unstable allele ($g^*$) produces a phenotype with pale yellow-green leaves and dark green sectors.

## 2. THE MUTATION OF $p^*$ IN THE SEPALS

The aim was to compare the rate of mutation to $p^b$ in sepals having two doses of $p^*$ ($p^*p^*$) with the rate in sepals with one dose ($p^lp^*$).

Because other genes may influence the mutation rate of $p^*$ an inbred stock was used. A $p^lp^*$ plant was self-pollinated, a $p^lp^*$ plant from among the progeny was self-pollinated and so on for four generations. In the fifth year the sepals of $p^lp^*$ and their sister $p^*p^*$ plants were chosen for examination by a predetermined scheme; the choice of sepals was uninfluenced by the number or size of their mutant areas. The upper epidermis was scanned under a microscope and the numbers of spots and sectors of 1, 2, 4, 8, . . . 1024 blue cells were scored. The procedure is essentially the same as that used by Demerec when he studied a similar mutable gene in *Delphinium ajacis* (Demerec 1931).

The average number of epidermal cells per square millimetre, based on 60 counts, was $756{\cdot}2 \pm 16{\cdot}3$ for $p^lp^*$ sepals and $849{\cdot}6 \pm 20{\cdot}0$ for $pp^{**}$ sepals. That the epidermal cells in $p^lp^*$ sepals were significantly larger than those in $p^*p^*$ sepals ($t = 3{\cdot}6$; $P = 0{\cdot}0005$) may be due to the obligate heterozygosity of the region of the chromosome marked by the $p$ factor in $p^lp^*$ plants.

From these data and a knowledge of the areas of the sepals that were scanned, it is possible to estimate the rate at which the $p^*$ gene mutates at successive cell

generations during the development of the sepals in both $p^l p^*$ and $p^* p^*$ plants (Tables 1 and 2). These estimates are the chance of mutation per $p^*$ gene per cell division of an unmutated cell. The number of unmutated cells at each cell generation can be found by subtracting those that would have mutated by that generation. In

Table 1. *Data on the mutation of* p* *to* p$^b$ *from* 30 *sepals of* p*p$^l$ *plants*

| Numbers of mutant ($p^b$) cells per spot | Numbers of spots observed | Numbers of divisions of unmutated cells. | Mutations per $p^*$ gene per cell division $\times 10^4$. |
|---|---|---|---|
| 1 | 781 | 1,028,072 | 7·60 |
| 2 | 516 | 514,294 | 10·03 |
| 4 | 349 | 257,321 | 13·56 |
| 8 | 242 | 128,782 | 18·79 |
| 16 | 160 | 64,471 | 24·82 |
| 32 | 92 | 32,281 | 28·50 |
| 64 | 53 | 16,167 | 32·78 |
| 128 | 29 | 8,098 | 35·81 |
| 256 | 20 | 4,059 | 49·27 |
| 512 | 11 | 2,035 | 54·05 |
| 1024 | 11 | 1,023 | 75·27 |
| > 1024 | 10 | 968 | 103·31 |

Table 2. *Data on the mutation of* p* *to* p$^b$ *from* 35 *sepals of* p*p* *plants*

| Number of mutant ($p^b$) cells per spot. | Numbers of spots observed. | Numbers of divisions of unmutated cells. | Mutations per $p^*$ gene per cell division $\times 10^4$. |
|---|---|---|---|
| 1 | 1911 | 1,172,821 | 8·15 |
| 2 | 1057 | 586,939 | 9·00 |
| 4 | 770 | 293,854 | 13·10 |
| 8 | 560 | 147,207 | 19·02 |
| 16 | 405 | 73,806 | 27·44 |
| 32 | 237 | 37,022 | 32·01 |
| 64 | 140 | 18,581 | 37·67 |
| 128 | 83 | 9,333 | 44·47 |
| 256 | 51 | 4,691 | 54·36 |
| 512 | 34 | 2,363 | 71·94 |
| 1024 | 18 | 1,190 | 75·63 |
| > 1024 | 26 | 1,139 | 124·18 |

making the estimates for $p^* p^*$ sepals the possibility of both $p^*$ genes in a cell mutating to $p^b$ has not been taken into account; it would have a negligible effect.

The data show that the mutation rate per $p^*$ gene per cell division is very similar in $p^l p^*$ and $p^* p^*$ flowers.

These results differ from those of Demerec (Demerec 1931) in two features. Whereas in Demerec's strain the rate of mutation per cell division of the unstable

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allele to the stable blue allele was constant throughout the development of the sepal, in the present strain the rate falls conspicuously during development. Second, Demerec did not record any evidence that the unstable allele was mutating to stable pink. In the present strain the occurrence of stable pink sectors, especially in $p^*p$ and $p^*p^l$ flowers, suggests such mutation. These two differences may be related. The continuing mutation of $p^*$ to stable pink will give a decreasing proportion of $p^*$ genes at each cell generation among the cells containing pink pigment. As stable pink cells cannot be distinguished from unmutated cells, the estimates of the mutation rate to $p^b$ would progressively fall even if the mutation rate of $p^*$ to $p^b$ were uninfluenced by the stage of development of the sepals. That it is not unlikely that both the mutation rate to $p^b$ and that to $p$ remain constant is suggested by the fact that the mutation rate to $p^b$ in both $p^*p^*$ and $p^*p^l$ sepals falls by an approximately constant amount, about 20%, at each cell generation. If this is the correct interpretation, $p^*$ must have a much higher mutation rate to $p$ than to $p^b$. In the next section, I shall show that this is so in the germinal tissues. I shall then also discuss just how stable these stable pinks really are.

An occasional sepal has a conspicuous sector with numerous small blue spots. It appears, to use McClintock's terminology, that $p^*$ has changed its state to one that only mutates to $p^b$ very late in the development of the sepals, and then at a very high rate. That the change is at $p^*$ has been confirmed by showing that this phenotype is inherited as if it were controlled by an allele of $p^l$ and $p^b$.

### 3. THE MUTATION OF $p^*$ IN GERMINAL TISSUE

By germinal tissue is meant the tissues from which pollen grains and embryo sacs are formed; it is not a line of cells distinct from the somatic tissue. If proof of this were needed it was provided in the previous paper (Dawson 1955) where blue flowers of a $p^*p$ plant were shown to produce a large proportion of $p^b$ pollen grains.

Table 3. *Progeny from reciprocal crosses between* p*p* *and* pp *and between* p*p$^l$ *and* pp

| Genotypes of parents | Number of crosses | Progeny: lavender $p^lp$ | unstable pink $p^*p$ | blue $p^bp$ | pink $pp$ |
|---|---|---|---|---|---|
| $pp \times p^*p^*$ | 10 | — | 435 | 2 | 11 |
| $p^*p^* \times pp$ | 34 | — | 1977 | 6 | 59 |
| $pp \times p^lp^*$ | 20 | 318 | 406 | 1 | 10 |
| $p^lp^* \times pp$ | 58 | 1597 | 1684 | 5 | 34 |

The first two lines of Table 3 set out the progeny that were obtained when $p^*p^*$ and $pp$ plants were crossed reciprocally. The majority of the progeny are $p^*p$; the two smaller groups $p^bp$ and $pp$ arise by the mutation of $p^*$ to $p^b$ and to $p$ respectively. In addition to these progeny there were four families from pollinating different inflorescences of a single $p^*p^*$ plant that together gave 137 $p^*p$ and 124 $pp$ offspring.

This plant was probably $p^*p$; a mutation to $p$ probably occurred in one of the gametes. The third and fourth lines set out the progeny from reciprocal crosses between $p^*p^l$ and $pp$.

The data show no evidence of different frequencies of mutant gametes among the embryo sacs and the pollen grains. Among 2490 $p^*$ gametes from $p^*p^*$ plants, 8 were $p^b$ mutants and 70 $p$ mutants. Among 2140 $p^*$ gametes from $p^*p^l$ plants, 6 were $p^b$ mutants and 44 $p$ mutants. Allowing for the fact that 31 of the 70 $p$ mutants from $p^*p^*$ plants were found in two families which together had only 130 progeny, the data are consistent with the rates of mutation per $p^*$ gene being the same in the germinal tissues of $p^*p^*$ and $p^*p^l$ plants. The data from the sepals allowed the calculation of rates of mutation per $p^*$ per cell division while the data from these crosses only provide estimates of the porportion of the gametes that contain mutant genes. However, it is important to note that even at the earliest stage of sepal development the proportion of $p^b$ mutant cells is not so low as is found among the gametes. This can only be explained if the rate of mutation is lower in germinal than in sepal tissue. Further, to explain the data from the sepals in terms of constant rates of mutation to $p^b$ and to $p$ throughout their development requires the mutation rate to $p$ to be between 10 and 20 times that to $p^b$. The data from the crosses suggest that the difference in the germinal tissue is about ten-fold. It is therefore possible that the relative rate of mutation of $p^*$ and $p^b$ and to $p$ is the same in sepal and germinal tissue and that both rates are lower in the latter than in the former.

Careful examination of the $pp$ progeny from these crosses showed that most had no sign of residual instability of the $p$ gene. There were, however, some plants which had a few sepals with a segment containing blue mutant sectors and spots. The segments were variable in appearance. In some the range of sizes of sectors and spots was similar to that in $p^*p$ plants while in others there was a dense 'dust' of small blue spots. The rarity of unstable segments among the sepals of some plants made it impossible to classify these $pp$ progeny with confidence into those which had, and those which did not have, residual instability. That this lack of confidence was justified was further demonstrated by self-pollinating eight of the $pp$ plants which showed no sign of instability in their sepals: six produced only stable pink progeny and two produced normal $p^*p$, or $p^*p^*$, and $pp$ progeny in approximate 3:1 ratios. Five other $pp$ plants were crossed to $p^lp^l$ and two gave only $p^lp$ progeny while three gave normal $p^*p^l$ and $p^lp$ progeny in approximate 1:1 ratios. It would seem that the apparent mutations of $p^*$ to $p$ are of two main types. The first has no detectable instability even after further crossing. The second has only temporarily lost its instability and usually regains it at some stage in the formation of the gametes, possibly during meiosis. The original $pp$ plants with segments of instability in a few sepals represent the occasional regaining of instability during the development of somatic tissues. That the second type of mutation does not always regain its instability at gamete formation is shown by the occurrence of the occasional plant among the progeny of a cross between a mutant $pp$ and $p^lp^l$ that has stable $p^lp$ sepals except for a rare sector with a few blue spots: the mutant $p$ gene has not regained its

instability until the development of the somatic tissues of the next generation. This was also clearly shown by the result of self-pollinating one of the *pp* mutants which had some sepals with segments of numerous small blue spots. Only few progeny from this selfing survived: one had all sepals with a dense 'dust' of blue spots, one was stable pink and the other two were pink with segments of small blue spots. The most striking example of mutant *p* genes regaining instability at gamete formation was provided by selfing one of the pink mutants, with no sign of instability in the sepals, that occur rarely in *p*p** stocks. The progeny from this stable pink plant were 49 unstable pink and only one apparently stable pink. A second such mutant from a *p*p** stock when crossed with $p^lp^l$ gave 16 $p^*p^l$ plants and 5 that had the phenotype of $p^lp$.

Examination of the $p^bp$ mutants revealed no sign of the back-mutation of $p^b$. Selfing these mutants and crossing them to *pp* produced only stable blue and stable pink offspring. There is no evidence of any residual instability in the $p^b$ mutant genes. The absence of unstable pink plants among the progeny of these mutants shows that chimaeras with a $p^bp$ epidermis over *p*p* tissue are rare, if they ever occur. This makes it unlikely that much, if any, of the data presented above on the occurrence of unstable pink progeny of apparently stable pink parents are to be interpreted in terms of the parents being chimaeras.

In the description of experiments in this and in the previous section I have noted one striking variant of the pattern of instability of *p** that has often occurred. This variant of *p** mutates at a high rate very late in the development of the sepals and so produces numerous small blue spots but hardly any large blue sectors. In the course of these and other experiments it has been possible to find and build stocks of another variant, which has the same range of sizes of blue spots and sectors as the main strain but the mutations occur much more rarely. Both these variants (and others could probably be selected) are inherited as alleles of the *p* factor.

### 4. ACTIVATOR OF *p**

All the observations that are presented in this paper are on a strain of *D. ajacis* that was originally obtained from Dr D. G. Catcheside. In this strain instability at the *p* locus was always inherited as an allele of $p^l$ and $p^b$. Later a strain was provided by the present Bishop of Montreal and from this a stable pink stock was selected. When this was crossed with the stable pink stock of the original strain all the progeny had sepals with blue spots and sectors. Complementary dominant genes are therefore necessary for instability to appear. One of these is *p**; the other can be designated *A* for activator. All my original stocks are presumably homozygous for *A*; the second stable pink stock is homozygous for *p** but lacks *A*.

### 5. INSTABILITY OF *g**

The recessive *g* gene first arose in a *p*p** stock. A subsequent family of *p*p** plants contained one plant which had fully green sectors on the yellow-green leaves. From this plant a stock was eventually obtained which when self-pollinated gave a

majority of offspring with mosaic leaves and a few which were fully green. The proportion of green offspring varied among the families from about 5% to up to about 50%. When these green plants, or wholly green branches from mosaic plants, were used in crosses, the mutant *G* gene was shown to have no detectable instability.

That *g** arose from *g* in a *p*p** plant suggests that the instability might have been transferred from *p** to *g*. The only other occasion on which *g** arose from *g* suggests this even more strongly. A plant that was known to be *p*pGg* was self-pollinated and produced 125 green, 22 yellow-green and 20 mosaic offspring. The *g* gene in the parent plant came from a *gg* stock that had previously shown no sign of instability: it had acquired instability in this plant. Unfortunately not all the progeny were grown to maturity and only 59 of the green plants could be scored for flower colour. That 31 were *p*p** or *p*p* and 28 were *pp* shows a remarkable excess of *pp* plants. It is therefore not unreasonable to suppose that many *pp* plants arose by *p** genes losing their instability to the *g* gene.

## 6. DISCUSSION

Those familiar with the work of McClintock on variegation (McClintock, 1948–1962) will have noticed many parallel observations in the present study: the existence of alleles with different patterns of instability, mutation to both stable and unstable alleles, the instability being shown in the presence of a dominant activator and circumstantial evidence of the transposition of instability from one gene to another. The close parallelism is sufficient to justify our interpreting the present data in terms of controlling elements.

The *p** gene has a controlling element attached at this locus. In the presence of the dominant activator this controlling element can induce the changes to *p* and $p^b$. The failure to find any residual instability in the $p^b$ mutant genes suggests that they arise by the transposition of the controlling element elsewhere in the genome: there is no evidence of its continuing presence at this locus. Either the *p** gene is structurally $p^b$ and transposition of the controlling element removes the suppression of its expression, or it is *p* and its mutation to $p^b$ is always accompanied by transposition of the controlling element to outside this locus. Similar alternatives will explain the apparently stable *p* mutants.

The mutations of *p** to alleles with different patterns of instability are most easily interpreted as transpositions of the controlling element to other sites within the locus: sites from which it transposes to give $p^b$ more, or less, readily at different stages of sepal development. If this is so the *p* mutants that later reacquire instability may be due to transpositions to sites within the locus (or even just outside it) from which transpositions rarely occur, or occur unaccompanied by mutation to a $p^b$ gene: the unstable sectors appear when further transpositions locate the controlling elements at sites from which they can readily transpose to give the $p^b$ mutants. That a considerable range of phenotypes with different patterns of instability is found among the progeny of these semi-stable pink plants is consistent with this explanation. It is possible that what McClintock calls a change of 'state'

may often, but not necessarily always, be a change of the site of attachment of the controlling element.

If the parallel with studies of instability in bacteria be admitted, there the instability of different unstable clones can be referred to different sites within the gene (Dawson & Smith-Keary, 1963; Smith-Keary & Dawson, 1964). Examples in bacteria of transpositions over short distances of the possibly similar elements, which we have called controlling episomes, appear to be fairly easy to find, and in some strains the frequency of such transposition can be very high indeed. It is therefore possible that transpositions of controlling elements over very short distances may account for some of the observations of gene instability in higher organisms.

I have discussed the data on the mutation of $p^*$ in the developing sepals and shown that the rate of mutation to $p^b$ per $p^*$ gene is the same in $p^*p$ as in $p^*p^*$ plants at any particular stage of development. The apparent fall in the rate per $p^*$ gene during development is reasonably interpreted as a spurious effect due to the decreasing proportion of $p^*$ genes in the tissue as $p^*$ mutates to $p$, also probably at a constant rate. The variant $p^*$ allele that only mutates to $p^b$ very late in development to give sepals with the appearance of a fine dust of blue spots also only mutates to $p$ late in sepal development: there are no large stable pink sectors. It looks as if the activity of the controlling element in producing $p^b$ mutants and its activity in producing $p$ mutants are closely related. The standard $p^*$ allele has both these activities throughout the development of the sepals while the variant $p^*$ is active in those physiological conditions that are only found during the later stages of sepal development. However, the previous work of Demerec (1931) indicates that a $p^*$ allele exists that mutates only to $p^b$.

The data on the origin of the yellow-green foliage mutant and the subsequent origin of its instability is too slight to permit any firm conclusions. What evidence there is points to the origin of $g$ from $G$ being related to the presence of $p^*$ and its subsequent instability to the transposition of the controlling element to it from $p^*$. This sequence has a parallel in a study of instability in the bacterium, *Salmonella typhimurium* (Dawson & Smith-Keary, 1963). A proline-requiring auxotroph was selected in a strain that was unstable at a leucine-suppressor locus (*su-leu A*), which may be the operator of the leucine operon. It was later shown that most clones that had become unstable for proline-requirement had lost their instability at *su-leu A*. While the instability for proline-requirement could not be shown to act at the same locus as contained the proline auxotroph mutation, rather than at a suppressor locus, the similarity with the present case in Delphinium is too close for it not be to mentioned.

McClintock has powerfully argued the similarities between the controlling element systems in maize and the operator-regulator system that Jacob has revealed in bacteria (McClintock 1961*a*, 1961*b*, 1962). That some controlling element systems are two-element systems strongly suggests this relationship. On the other hand, the similarity of the patterns of instability in bacteria to those in maize suggests that there may be an even closer relationship between controlling elements

and controlling episomes. The ability to refer the transposable units in bacteria to particular sites within a gene suggests that they are not whole operator or regulator regions. Until we know much more about the behaviour of these different systems it may be wise to keep separate the terms operator-regulator, controlling element system and controlling episome.

## SUMMARY

1. Inheritance of flower colour in *Delphinium ajacis* is controlled by a locus with three stable alleles: $p^b$ (blue) is dominant to $p^l$ (lavender), which is dominant to $p$ (pink). An unstable allele, $p^*$, exists in a number of states, distinguished by the pattern of their mutation to $p^b$ and $p$ during the development of the sepals.

2. The rate of occurrence of blue sectors and spots in the developing sepals of $p^*p^*$ plants is twice that in the sepals of $p^*p^l$ plants. The rate of mutation to $p^b$ apparently falls during the development of the sepals and this is probably due to $p^*$ mutating also to the stable $p$ allele.

3. The frequency of $p^b$ and $p$ gametes from $p^*p^*$ plants is twice that from $p^*p^l$ plants.

4. The $p^b$ mutants from $p^*$ show no evidence of instability; the controlling element has transposed from the locus.

5. Some $p$ mutants from $p^*$ are apparently stable; others can reacquire instability, especially during sexual reproduction. The latter are tentatively interpreted as due to transpositions of the controlling element to other sites within the gene.

6. The instability of $p^*$ is only shown in the presence of a dominant activator.

7. Evidence is presented of transposition of the controlling element from $p^*$ to a locus controlling the colour of the foliage.

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