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# Dosage dependence of maternal contribution to somatic cell division in *Drosophila melanogaster*

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## Summary

**Most mitotic mutants in *Drosophila* do not lead to lethality in early development despite the highly abnormal chromosome behaviour that they elicit. This has been explained as being the effect of maternally provided wild-type products. We have tested this hypothesis by studying cuticular clones derived from cells in which there has been loss of a marked *Y* chromosome due to chromosome nondisjunction in individuals homozygous for the mutation *abnormal spindle* who are progeny of heterozygous mothers. We have found that the size and frequency of these clones are higher than in control flies. Furthermore, by analysing flies whose female parents have different doses of the $asp^+$ gene, we have found that there is a correlation between the amount of maternally contributed $asp^+$ product and the frequency and size of cuticular clones. We have also estimated the time in development when the first mitotic mistakes take place, i.e. the time when maternal products are no longer sufficient to carry out normal cell division.**

Key words: abnormal spindle, somatic nondisjunction, cell division, maternal effect.

## Introduction

The genetic analysis of cell division in *Drosophila* has identified a large number of loci involved in this process (Gatti *et al.* 1983; Ripoll *et al.* 1987; Gatti and Baker, 1989). An interesting feature of many of these mutants is that they do not cause lethality early in development, but rather homozygous larvae survive to the late larval/early pupal period or even to adulthood. It has been proposed that this delay in the lethal effective phase results from the presence in the oocyte of enough wild-type products provided by the mother to complete the embryonic divisions required for production of larval tissues (Ripoll, 1977; Baker *et al.* 1982; Szabad and Bryant, 1982; Taubert and Szabad, 1987). The zygotic genotype should affect only those tissues that remain diploid (among them imaginal cells) and then only when the maternal supply is not enough to maintain normal cell division (Gatti *et al.* 1983; Ripoll *et al.* 1987; Gatti and Baker, 1989).

The maternal contribution to development has been the subject of intensive studies in a wide range of organisms. In *Drosophila*, both classical and molecular genetic approaches have aimed to identify those maternal genes whose products play a key role in development (Nusslein-Volhard *et al.* 1987; Akam, 1987; Ingham, 1988). Except for those functions specifically involved in very early stages of embryogenesis, for which the only source of gene products is the maternal one, zygotic products gradually substitute for the maternal ones when zygotic transcription starts after the blastodermal stage (McKnight and Miller, 1976).

Molecular analysis has, until now, been unable to determine the time at which the maternal–zygotic transition takes place, since the presence of a transcript or protein by no means implies functionality. To know when the maternally provided products are no longer able to carry out their function and the zygotic ones begin to be essential requires a functional assay. Only classical genetics can at this moment – and only in cases when mutant phenotypes allow this kind of analysis – address this question. One such analysis was carried out by Robbins (1984) making use of mutants in the *zeste-white* region. We have carried out such an analysis with mutations in the gene *abnormal spindle* (*asp*, Ripoll *et al.* 1985). One of the phenotypes shown by somatic cells in *asp* individuals is a high frequency of chromosome nondisjunction. We have studied the kinetics of chromosome nondisjunction by analysing cuticular clones (Postlethwait, 1978) produced by the loss of a marked *Y* chromosome. To this aim, we have taken advantage of cuticular cell markers (mutations that mark cells without interfering with their normal

development) whose wild-type alleles have been translocated to the *Y* chromosome. Cells that are aneuploid for the *Y* chromosome are viable since the lack or excess of *Y*-chromosome material is irrelevant for viability (Bridges, 1916). However, the loss of a *Y* chromosome that carries a wild-type copy of a cuticular marker will result in the appearance of cuticular spots displaying the mutant phenotype. Thus, it is possible to study the mitotic instability of this *Y* duplication in *asp* flies, and by analysing the sizes and frequencies of the cuticular clones information can be gained on how the mutant tissue grows and when the zygotic product is essential for normal cell division.

To determine the extent of the maternal contribution to the expressivity of the mutant phenotype in *asp* flies, we have extended this analysis to individuals derived from mothers bearing different doses of the $asp^+$ gene. Our results show that the size and frequencies of cuticular clones (indicative of the time in development when the zygotic products predominate over maternal ones) are correlated with the number of wild-type doses of the gene carried by the maternal genome.

## Materials and methods

### *Drosophila strains*

All the mutations and rearrangements used in this work are described by Lindsley and Grell (1968) with the following exceptions: the alleles of *abnormal spindle* (Ripoll *et al.* 1985; Gonzalez *et al.* 1989*b*); $Dp(3;3)M96C^{2+}9$, a tandem duplication for the $asp^+$ locus (Gonzalez *et al.* 1989*a*); *C(3)EN, th st,* a rearrangement in which a pair of third chromosomes share a single centromere (Novitski *et al.* 1981); and *TM6B,Hu e Tb ca*, a multiply inverted chromosome (Craymer, 1984). Since the severity of the mitotic phenotype of *asp* individuals has been shown to be temperature-dependent, flies were reared on standard *Drosophila* medium at 17 or 29°C as indicated in each case.

### *Allelic combination*

These experiments can only be performed following careful choice of the proper combination of mutant alleles. Ideally, viability should be high enough to enable the study of cuticular clones in adult tissues, and yet the phenotype must be penetrant enough to be easy to quantify. From our collection of *asp* alleles (Gonzalez *et al.* 1989*b*), we have chosen the combination $asp^{E3}/asp$. The frequency of aneuploid cells in $asp^{E3}/asp$ is slightly higher than in either *asp* or $asp^{E3}$ homozygous larvae, but the heterozygote is more viable than either of the homozygous lines. The heterozygote $asp^{E3}/asp$ is henceforth called simply *asp*.

Four different experiments have been carried out in this work. We have analysed *yellow* (*y*) and *Hairy wing* (*Hw*) clones in *asp* flies derived from $asp^{E3}/+$ mothers and *multiple wing hairs* (*mwh*) clones in *asp* flies obtained from mothers with varying doses of the *asp* gene, namely: $asp^{E3}/+$, $+/+$, and $asp^{E3}/+/+$. These flies were obtained as follows:

(1) *yellow* and *Hairy wing* clones in asp flies produced by $asp^{E3}/+$ mothers. $y/y^+Y$;*red asp/TM6B,Hu e Tb ca* males were crossed to *y;e* $asp^{E3}$*/TM6B,Hu e Tb ca* females to produce $y/y^+Y$;*red asp/e* $asp^{E3}$ (*asp*) males. Their $y/y^+Y$;*red asp/TM6B,Hu e Tb ca* siblings were used as a control.

(2) *multiple wing hairs* clones in asp flies produced by $asp^{E3}/+$ mothers. *y;*$Dp(1;3)sc^{J4}$,$y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$*/TM6B,Hu e Tb ca* females were crossed to both *y/* $Dp(3;Y)D8,y^+mwh^+$; *mwh jv tra* $p^p$ *red asp/TM6B, Hu e Tb ca* and *y/*$Dp(3;Y)D8,y^+mwh^+$; *mwh e ro ca/TM6B, Hu e Tb ca* males to obtain *y/*$Dp(3;Y)D8,y^+mwh^+$;*mwh jv tra* $p^p$ *red asp/*$Dp(1;3)sc^{J4}$, $y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$ (*asp*) and *y/*$Dp(3;Y)D8,y^+mwh^+$;*mwh e ro ca/*$Dp(1;3)sc^{J4}$,$y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$ (control) males respectively. Both of them are homozygous for *mwh* and carry a $mwh^+$ duplication on the *Y* chromosome.

(3) *multiple wing hair* clones in asp flies produced by $asp^+$ mothers. To obtain *asp* flies from wild-type ($+/+$) females we used a *C(3)EN* chromosome. This rearrangement is composed of a pair of third chromosomes joined together to a single centromere (Novitski *et al.* 1981). As a result females bearing this rearrangement produce either nullo-third or diplo-third chromosome gametes which only give rise to viable progeny when fertilised by males producing the complementary aneuploid sperm. Homozygous *asp* males have been shown to produce aneuploid sperm due to abnormal chromosome segregation during both meiotic divisions and consequently give a high number of descendants when mated to *C(3)EN* females (Gonzalez, 1986). Males of the genotype *y/*$Dp(3;Y)D8,y^+mwh^+$;*mwh jv tra* $p^p$ *red asp/*$Dp(1;3)sc^{J4}$, $y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$, obtained as described in the previous paragraph, were crossed with *y;C(3)EN,th st* females. From the progeny of this cross, only *asp* males with the same genotype as their fathers were studied (nullo-third oocytes fertilised by diplo-third sperm produced by nondisjunction during the first meiotic division). Since *asp* males are sterile at 17°C this experiment was only carried out at 29°C. No control flies were included since $asp/+$ males do not produce progeny when mated to *y;C(3)EN* flies.

(4) *multiple wing hairs* clones in asp flies produced by $asp^{E3}/+/+$ mothers. The mutation $asp^{E3}$ has been shown to be hypomorphic: the mitotic abnormalities shown by $asp^{E3}$ homozygous flies are slightly weaker than those found in flies heteroallelic for this mutation and a deficiency that uncovers the *asp* gene (Gonzalez, 1986). This means that it partially performs the function of the wild-type allele and thereby females of genotype $asp^{E3}/+/+$ must contribute to the oocyte an additional amount of partially functional product relative to a wild-type female ($+/+$). To examine the extent of the contribution of this extra dose of the *asp* function, we used females carrying $asp^{E3}$ in *trans* with a tandem duplication for the wild-type allele of *asp*. Females *y;mwh red* $Dp(3;3)M96C^{2+}9/Dp(1;3)sc^{J4},y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$ were crossed to both *y/*$Dp(3;Y)D8,y^+mwh^+$; *mwh jv tra* $p^p$ *red asp/TM6B, Hu e Tb ca* and *y/*$Dp(3;Y)D8,y^+mwh^+$; *mwh e ro ca/TM6B, Hu e Tb ca* males to obtain *y/* $Dp(3;Y)D8,y^+mwh^+$;*mwh jv tra* $p^p$ *red asp/*$Dp(1;3)sc^{J4}$,$y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$ (*asp*) and *y/*$Dp(3;Y)D8,y^+mwh^+$;*mwh e ro ca/*$Dp(1;3)sc^{J4}$,$y^+$ $sc^{J4}$ *mwh* $Sb^{63}$ *e* $asp^{E3}$ (control) males respectively. Both of them are homozygous for *mwh* and carry a $mwh^+$ duplication in the *Y* chromosome.

### *Clone analysis*

Cuticles were opened under the dissecting microscope, cooked in KOH to remove soft parts, washed in alcohol and mounted in Euparal. Wings were dissected, washed in alcohol and mounted in Euparal. Observations were made under the compound microscope. *yellow* clones were analysed in heads, thoraces, abdomens and legs, and *multiple wing hairs* and *Hairy wing* phenotypes were examined in wings.

## Results

One of the consequences of mutations in *asp* is an alteration in the mechanisms of chromosome segregation so that cells undergoing mitosis frequently produce aneuploid descendants. In the following experiments, we have studied the occurrence of nondisjunction of *Y* chromosomes that were labelled with wild-type copies of either *yellow* or *multiple wing hairs*. Fig. 1 illustrates the production of clones in these flies and their phenotypes.

### yellow *clones in* asp *flies produced by* asp/+ *mothers*

The first experiment to study the mitotic stability of the *Y* chromosome in *asp* flies was carried out in males that were mutant for *y* and carried a $y^+Y$ duplication. The presence of this duplication has two effects. Firstly it rescues mutations in *y*, although inactivation of the $y^+$ gene in some cells due to position effect variegation (Spofford, 1976) brought about by the heterochromatic *Y* chromosome will result in the appearance of some phenotypically *y* cells. Secondly, it produces a dominant *Hairy wing* phenotype which consists of the appearance of extra bristles on wings, head and thorax. This effect is dosage-dependent: one dose of $y^+Y$ produces few extra bristles whereas two doses produce many (Williamson, 1968). The loss of the $y^+Y$ chromosome in some cells will thereby lead to the production of *y* clones in a $y^+$ background, whereas more than one copy of this duplication will result in an increased *Hw* phenotype. In this experiment, both *y* and *Hw* phenotypes were quantified.

The results are shown in Fig. 2. Both *yellow* and *Hw* bristles are significantly more frequent in *asp* than in control males (Fig. 2A,B). The level of both *y* and *Hw* clones in control flies is well above zero and shows a clear temperature-dependence; the lower the temperature, the more *y* and *Hw* spots produced. Despite this relatively high background level, the different nature of the *y* clones present in *asp* and control flies is substantiated by the distribution of clone sizes (Fig. 2C). No clones with more than two bristles were found in control flies, and in fact more than 90 % of the clones are accounted for by single bristles, whereas clones containing more than ten bristles were observed in their mutant siblings.

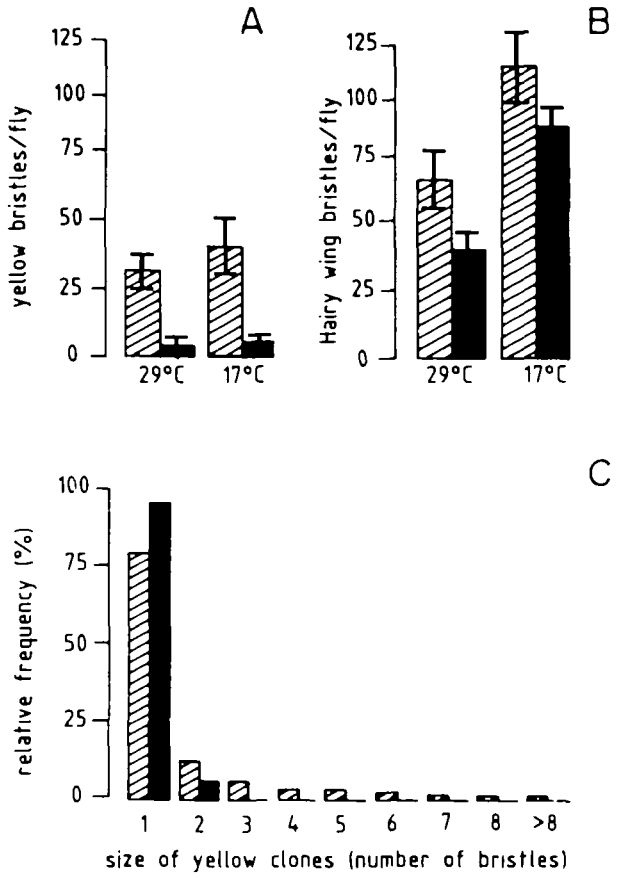


**Fig. 2.** Production of *y* and *Hw* clones in *asp* flies. (A) *y* and (B) *Hw* bristles per fly in *asp* (dashed) and control (black) males reared at 29 and 17°C. (C) size distribution of *y* clones in *asp* and control flies reared at 29°C. In each case, twenty flies were scored for *y* and *Hw* clones. The bar indicates the standard deviation.

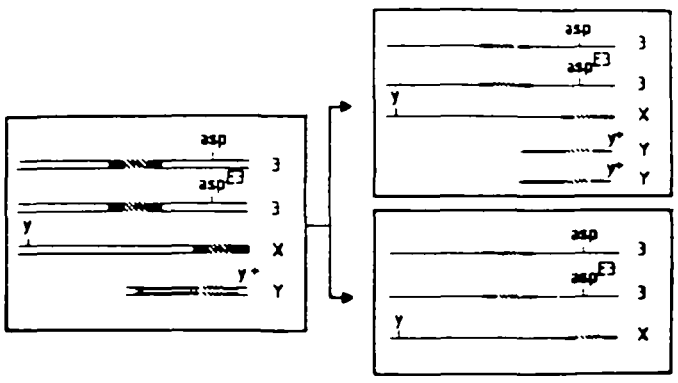


**Fig. 1.** Scheme of a clone-originating event by nondisjunction of a marked *Y* chromosome. The cell on the left is mutant for *y* and *asp* and carries a $y^+$ duplication on the *Y* chromosome. If mitotic nondisjunction of the *Y* chromosome occurs, one of the daughter cells will receive two *Y* chromosomes (top) whereas the other will be nullo-*Y* (bottom). The latter lacks the wild-type allele of *y* and therefore its offspring will show this phenotype. The genotypes depicted in this figure do not exactly correspond to the ones used in this work. For a description of the actual genotypes involved on each experiment see 'Materials and methods'.

We interpret these results as an indication of the mitotic instability of the $y^+Y$ duplication due to the *asp* mutation which leads to gain as well as to loss of this chromosome. We can discard a mutational effect of *asp* as the source of *y* clones because no such clones were found in homozygous ($y^+/Y;asp/asp$), heterozygous ($y^+/Y;asp/+$) and control ($y^+/Y;+/+$) males, where the only source of *y* clones is mutagenesis of the X-linked wild-type copy of this gene (20 flies of each genotype scored). An alternative explanation for the increase of *y* clones in *asp* flies would be that mutation in this gene results in some sort of variegation-

enhancing activity, thus increasing the frequency of cuticular spots already present in control flies. One of the purposes of the following experiment was to discard variegation effects as an alternative interpretation.

*mwh clones in* asp *flies: effect of the maternal contribution of* asp$^+$ *doses*

We designed a series of experiments similar to those described above, but using a $mwh^+Y$ duplication. The loss of this chromosome in *mwh* flies leads to the production of *mwh* clones. *mwh* transforms the single hair present on each wing cell into a tuft of two to five hairs (Lindsley and Grell, 1968). Compared to the previous experiment based on the analysis of *y* clones, this approach has several advantages. Firstly, the $mwh^+Y$ duplication shows no variegation effects. Secondly, as one hair is produced by each cell of the wing blade in wild-type flies (Garcia-Bellido and Merriam, 1971), the study of *mwh* clones allows the identification of phenotypic changes in single cells. Finally, clonally related cells remain together during the development of the wing disc (Garcia-Bellido and Merriam, 1971). These features enable a more precise quantification of the phenomenon under study to be made, thus facilitating an estimate of the actual contribution of the maternal genome.

Indeed, maternally provided $asp^+$ product is the best candidate for the delay in the appearance of the mutant phenotype in asp flies, but to prove it, the correlation must be made between the number of $asp^+$ doses provided by the mother and the kinetics of the production of clones. To this end we performed the following experiments with males derived from females bearing different doses of the *asp* gene. The schemes used to obtain these flies are detailed in 'Materials and methods'. In the first case, $mwh^+Y$; *mwh asp/mwh* $asp^{E3}$ males were derived from $asp^{E3}/+$ females. In a second experiment, the males were originated from *C(3)EN* females ($asp^+/asp^+$), so that the maternal dose of functional *asp* product contributed by the mother was greater. Finally we analysed males derived from $Dp(3;3)M96^{c2+}9, asp^+/asp^{E3}$ females. Hence, as far as the maternally contributed doses of *asp* are concerned, *asp* males from (1) $asp^{E3}/+$; *(2)* $+/+$ *and (3)* $asp^{E3}/+/+$ mothers were obtained for clonal analysis.

The results of all these experiments are presented together in Table 1 and plotted in Fig. 3. The number of clones in control males is very low and their average sizes are small and similar to those reported previously (Baker *et al.* 1978; Kennison and Ripoll, 1981). Neither their frequency nor their size are temperature-dependent. On the other hand, *asp* flies have larger and more numerous clones per wing than control flies, regardless of the maternal genotype and the temperature at which the experiment was carried out. The production of clones in *asp* flies shows some temperature-dependence: clones are more frequent and their sizes are on average slightly smaller at 17 °C. We interpret this result to be a consequence of the temperature-sensitivity of this allelic combination as revealed by cytological analysis of mitotic figures in the ganglia of third instar larvae: the lower the temperature the higher the

**Table 1.** *The effect of temperature and maternal contribution of* asp$^+$ *product on the production of* mwh *clones* in* asp *(E) and control (c) males*

| | | Maternal genotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | +/asp | | | | +/+ | +/+/asp | | | |
| | | Incubation temperature (°C) | | | | | | | | |
| | | 29 | | 17 | | 29 | 29 | | 17 | |
| Number of divisions | Number of cells per clone | E | C | E | C | E | E | C | E | C |
| 1 | 1 | 48 | | 83 | 1 | 165 | 344 | 6 | 470 | 11 |
| 2 | 2 | 39 | | 72 | | 129 | 218 | 1 | 299 | 2 |
| 3 | 3–4 | 55 | | 70 | | 148 | 196 | 1 | 258 | |
| 4 | 5–8 | 67 | 1 | 62 | | 153 | 146 | | 170 | |
| 5 | 9–16 | 35 | | 58 | | 100 | 96 | | 99 | |
| 6 | 17–32 | 26 | | 31 | | 60 | 38 | | 47 | |
| 7 | 33–64 | 17 | | 12 | | 29 | 11 | | 19 | |
| 8 | 65–128 | 1 | | 10 | | 12 | | | | |
| 9 | 129–256 | 3 | | 3 | | | | | | |
| 10 | 257–512 | 1 | | | | | | | | |
| | Total | 292 | 1 | 401 | 1 | 796 | 1049 | 8 | 1362 | 13 |
| | Clone size | 11.4 | 7.0 | 10.5 | 1.0 | 8.4 | 4.5 | 1.3 | 4.5 | 1.2 |
| | Max. clone size | 300 | 7 | 188 | 1 | 101 | 61 | 3 | 60 | 2 |

Flies were obtained from mothers with different doses of the *asp* gene

(+/asp) *y; Dp(1;3)sc$^{14}$ mwh Sb$^{63}$ e asp$^{E3}$/TM6B*

(+/+) *y; C(3)EN, th st*

(+/+/asp) *y; mwh red Dp(3;3)M96C$^{2+}$9/Dp(1;3)sc$^{14}$ mwh Sb$^{63}$ e asp$^{E3}$*

(E) *y/Dp(3;Y)D8, mwh$^+$ y$^+$; mwh jv tra p$^P$ red asp/Dp(1;3)sc$^{14}$ mwh Sb$^{63}$ e asp$^{E3}$*

(C) *y/Dp(3;Y)D8, mwh$^+$ y$^+$; mwh e ro ca/Dp(1;3)sc$^{14}$ mwh Sb$^{63}$ e asp$^{E3}$*

* In each case, ten wings were scored for *mwh* clones.

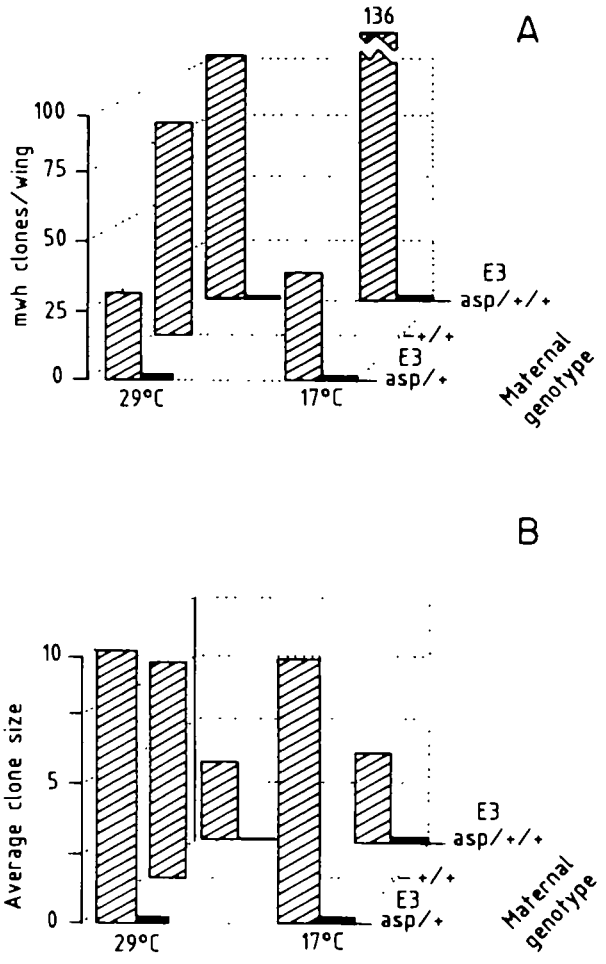


**Fig. 3.** Effect of temperature and the number of maternal doses of *asp* in the frequency and size of *multiple wing hair* clones. The figures shown in Table 1 have been plotted to illustrate the effect of temperature and maternal genotype on the number of clones per wing (A) and the average clone size (B). Flies were obtained from mothers with different doses of *asp* and reared at 17 and 29°C as indicated. In the case of flies derived from +/+ mothers control flies cannot be obtained, nor can the experiment be carried out at 17°C. hatched bars, *asp*; black bars, control.

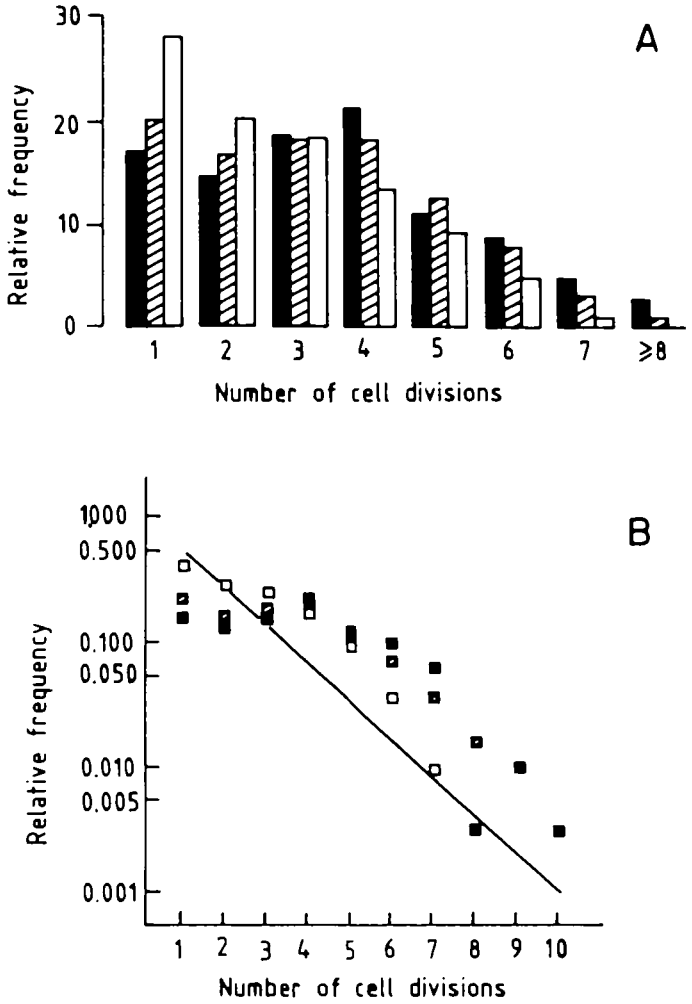


**Fig. 4.** Effect of the maternal genotype on the distribution of sizes of *multiple wing hair* clones in *asp* males. The figures shown in Table 1 have been plotted to illustrate the effect of the maternal genotype on the distribution of clone sizes. (A) Histogram of the distribution of frequencies of clones of different sizes *versus* the number of cell divisions required for them to be produced. (B) Relative frequency (logarithmic scale) of clones of different sizes *versus* number of cell divisions. The straight line shows the distribution expected in a normal, exponentially growing cell population where the probability of the production of a clone is the same at any cell division. Males were obtained from $asp^{E3}/+$ (black), +/+ (hatched), and $asp^{E3}/+/+$ (white) mothers. Only the results concerning flies reared at 29°C have been included.

frequency of cell cycle arrest and polyploid cells in $asp/asp^{E3}$ individuals.

As far as the effect of maternally contributed *asp* product on the production of clones is concerned, the results shown in Table 1 and Fig. 3 indicate that, at both temperatures (17°C and 29°C), the increase in the amount of maternal *asp* product from $asp^{E3}/+$ to $asp^{E3}/+/+$ results in both an increase in the number of *mwh* clones per wing (from around 30 to more than 100) and a decrease in the average clone size (from 11 to 4 cells per clone). From the data presented in Table 1 it is evident that these two effects are clearly interrelated in as much as the increase in the number of clones is mainly contributed by small ones, hence there is an overall decrease in the average clone size.

This is better illustrated in Fig. 4A,B where we have plotted the frequency distribution of clones of a given size. Assuming that growth is exponential (every cell division doubles the number of cells in the disc) and that every cell has the same probability of giving rise to a *mwh* clone throughout development, smaller clones would be more frequent than larger ones and the relation between clone sizes, expressed as the number of cell divisions required for them to appear and the logarithm of their relative frequency, would be a straight line. As shown in Fig. 4A,B *asp* individuals produced by $asp^{E3}/+$ mothers show a distribution of clone sizes that clearly departs from the expected one.

Smaller clones are not always more frequent than larger ones: clones that require 1, 2 and 3 cell divisions to be generated are less frequent than those that require 4 cell divisions. Consequently, the relationship between clone sizes and the logarithm of their relative frequencies is not a straight line. Nevertheless, these two plots become closer to the expected in the progeny of +/+ females, and even more so in the progeny of $asp^{E3}/+/+$ females.

Two conclusions can be drawn from these data. Firstly, at least one of the aforementioned assumptions is not fulfilled by *asp* males: either their wing imaginal cells do not grow as wild type or the probability that those cells give rise to a $mwh^+$ clone is not constant throughout development, or both. Second, the abnormal distribution of clone sizes produced by mutation in *asp* is partially circumvented by increasing the number of $asp^+$ doses provided by the mother: the more maternally contributed $asp^+$ product, the closer the distributions are to the expected, assuming exponential cell proliferation and identical probability of clone formation at each generation. We interpret these results as a direct proof of the effect of maternal contribution of the *asp* function on the production of clones.

Additional evidence in favour of this interpretation comes from the analysis of the effect of maternal dose on the maximum clone size. The size of a clone gives information about the division in which it was formed and consequently the maximum clone size indicates the time in development at which alterations of cell division first occur. In the case of *asp* males originating from $asp^{E3}/+$ mothers, the largest clone comprised 300 cells. Ten cell divisions are needed for such a clone to be produced, which means it was formed 64 to 72 h before puparium formation, i.e. during the first half of the second larval instar (Garcia-Bellido and Merriam, 1971). Among the males coming from +/+ mothers, the largest clone that we observed had 101 cells. Such a clone necessitates eight cell divisions for its formation, meaning that it originated 56 to 64 h before puparium formation (Garcia-Bellido and Merriam, 1971). This corresponds to a delay in the expression of the mutant phenotype of two cell divisions later than in the previous experiment. Finally, when *asp* males were derived from $asp^{E3}/+/+$ mothers, the largest clone contained 61 cells indicating that the first loss of the *Y* chromosome took place in the seventh division before the end of the disc growth, one division later than in the second experiment. Therefore, the time in development at which the first errors in *Y* chromosome disjunction take place depends on the amount of maternally provided $asp^+$ product.

## Discussion

Many of the mitotic mutants described to date in *Drosophila* do not produce early lethality, despite the highly abnormal pattern of chromosome behaviour that they present (Gatti *et al.* 1983; Ripoll *et al.* 1987). This has been thought to be due to the effect of maternally supplied wild-type products since they assure normal divisions in early embryogenesis, when most of the mitotic activity for producing the larvae itself takes place.

In this study, we have sought to analyse the extent of the maternal contribution to the development of *asp* flies. Among the phenotypic traits characteristic of mutations in *asp* (Ripoll *et al.* 1985), the production of aneuploid cells offers the easiest way to address this problem. Firstly, it is easy to quantify: the frequency of aneuploid cells and the degree of aneuploidy can be scored in cytological preparations of the ganglia of third instar larvae. Secondly, it is amenable to clonal analysis, i.e. in flies homozygous for a cell marker, the loss of a duplication that covers that region brings about that particular phenotype. By scoring clones originating from nondisjunction of a marked chromosome, it is possible to estimate the time at which alterations to cell division take place in the development of a mutant individual. Thus, it is possible to correlate the production of aneuploid cells, as seen in cytological preparations, with the production of cuticular clones.

The *Y* chromosome offers an ideal tool for such a purpose since neither its loss nor its gain have any effect on viability (Bridges, 1916), and there are many insertions from euchromatic regions that cover mutations in cell marker genes. Although this approach only takes into account a small fraction of the types of aberrant chromosome segregation in *asp* flies (loss or gain of the *Y* chromosome), it offers an easy and quantifiable estimate of such effects. We have used two different markers: $y^+Y$ and $mwh^+Y$. Although the results obtained with *mwh* are more easily quantified and are free of the effects of variegation, the $y^+Y$ chromosome allows for the study of gain of function in clonal experiments so that duplication, as well as loss, of the *Y* chromosome can be scored.

From the different alleles of *asp* available, we have chosen for these experiments the hypomorphic heteroallelic combination $asp/asp^{E3}$. There are two reasons for doing so. Firstly, perdurance of maternal products is independent of whether or not the zygotic alleles are null or make some product. Secondly, being hypomorphic, the products from these two alleles are able partially to perform the wild-type *asp* function and individuals carrying this combination show high levels of chromosome nondisjunction and relatively high viability. More amorphic combinations result in a higher proportion of cells being arrested at metaphase and lower viability, thus making this kind of study much more difficult.

The results obtained with both duplications indicate that the kinetics of the appearance of cuticular spots (i.e. nondisjunction of the marked Y chromosome) is significantly different in *asp* compared with control flies. The former showed more numerous and, on average, larger clones than their wild- type siblings. We have also shown that this production of cuticular spots in *asp* flies is directly dependent upon the amount of functional *asp* product provided by the mother; the more maternally contributed product, the more fre-

quent and smaller these clones are. We interpret these results to be direct proof of the link between maternal contribution and manifestation of the mutant phenotype.

The actual effect of the maternal products on the kinetics of clone production in *asp* flies has two components. Firstly, for large clone sizes (those corresponding to clones produced at or before four cell divisions before the end of disc growth), increasing maternally provided *asp* product results in a delay in the appearance of cuticular spots; the higher the maternal contribution, the lower the relative frequency of clones of a given size. Secondly, for small clones (generated 1,2 or 3 cell divisions before the end of disc growth) their relative frequency is increased by increasing the maternal contribution.

The reduction in large clones caused by increasing the amount of maternally provided product can be readily explained as the result of the increase of the perdurance of that function: the more maternally contributed product, the longer it lasts, and therefore the later chromosome nondisjunction starts to occur and fewer large clones are produced. It is not so obvious, though, why an increase in the perdurance of the *asp* function should result in an overall increase in the number of small clones produced. Theoretically, if the number of cells double each generation and the probability that each cell gives rise to a clone through development is constant, the frequency distribution of clone sizes is such that small clones are more frequent than larger ones (the probability that a clone will appear late in development is larger because there are more cells), and the plot of the logarithm of frequency *versus* size is a straight line (as a result of the exponential kinetics of cell proliferation). The kinetics of clone production by X-ray-induced mitotic recombination fulfills these expectations (Garcia-Bellido and Merriam, 1971).

Although we do not understand why the distribution of clone sizes in *asp* flies departs from the theoretical one, we would like to point out the following considerations. Firstly, unlike the case of X-ray-induced clones which are produced only at the time in development when the flies are irradiated, the potential for the generation of clones in *asp* flies (i.e. mutation in *asp*) is present throughout development. Secondly, cell proliferation cannot be exponential in these flies whose cells show high levels of aneuploidy and cell cycle arrest. Thirdly, due to the maternal effect shown in this work, the probability that a clone-generating event will happen can hardly be expected to be constant throughout development. These three considerations lead one to expect that the kinetics of clone production in *asp* cells would differ greatly from that induced by X-rays.

A number of other factors may also contribute to the abnormal distribution of clone sizes in *asp* flies. For instance the deleterious effects of aneuploidy for chromosomes other than the *Y* (Ripoll, 1977) will result in cell death within and outside marked clones, thus contributing to this effect. Similar consequences will result from the effect of the cell cycle arrest brought about by mutation in *asp*. Furthermore, dead cells within a clone can be replaced by healthy unmarked ones, therefore introducing another distorting factor. Nondisjunction of the *Y* chromosome during early stages of development will decrease the number of cells with one *Y* chromosome in later stages, hence decreasing the probabilities that small clones will be produced, etc. All these considerations, and perhaps others, must contribute to some extent to the fact that the kinetics of clone production in mutant *asp* discs departs in many ways from a theoretical situation in which clones are induced in wild- type cells by means of an external agent. In this system the cause for clone generation is mutation in *asp*, which at the same time affects cell proliferation and whose expressivity is variable through development.

As with the other parameters, the largest clone size is also dependent upon the amount of maternally provided product. Increasing the amount of maternally contributed $asp^+$ function results in a delay in the appearance of the first clone. Genotypically identical mutant males derived from $asp^{E3}/+$, $+/+$, and $asp^{E3}/+/+$ show largest clones which, according to their sizes, should have been generated ten, eight, and seven cell divisions before the end of wing disc growth, respectively. A similar conclusion is reached when, instead of considering the largest clone size, which of necessity is based on the rarest event and thus strongly dependent upon sample size, one considers the transition from no clones to a measurable number. These results indicate that the degree of perdurance correlates with the dose of maternally provided gene product; the more the maternal contribution, the later the mistakes in chromosome segregation begin. The fact that an increase in the maternally contributed product of just one dose of the hypomorphic allele $asp^{E3}$ has some effect on the time at which the first clone is produced indicates that, in the case of *asp*, this kind of analysis is sensitive to levels of gene activity which are lower than one dose of the wild-type gene. Although these two parameters (the largest clone size, and the transition from zero to some clones) offer a unique and straightforward way of estimating the time at which detectable mitotic alterations begin, the following considerations have to be made. Firstly, due to the alterations in the kinetics of cell proliferation in *asp* flies discussed above, the size of a clone in these flies might not exactly reflect the time in development at which the clone was produced. Secondly, it should be emphasised that the maximum clone size does not necessarily reflect the time at which the maternal product disappears; rather it probably reflects the time at which the wild-type function falls below a critical level.

Our study of the effect of the maternal contribution on the expressivity of the mutant phenotype has been limited to the wing. It is expected that other cell lineages, in which the number of cell divisions or the time at which the bulk of mitotic activity takes place are different, will show different parameters of clone production. We do not know whether the decay of the

perdurance of the maternal products is due to the limited half-life of the product, to dilution through cell division, or to both. By extending the kind of analysis presented in this work to other tissues it should be possible to address this point.

In this work we have shown that maternal $asp^+$ product can influence the development of mutant animals until surprisingly late. We do not know how general this phenomenon might be, but there is no reason to believe that the case that we have studied is exceptional. A similar result was obtained by Baker *et al.* (1978). These authors reported that the sensitivity of *mei-9* and *mei-41* mutant males to ultra-violet irradiation was much lower, and at about wild-type levels, in larvae irradiated during the first instar than in those treated as third instar. Although no studies of the effect of gene dosage were carried out in this case, these authors put forward the hypothesis that maternal product could be responsible for rescuing the UV sensitivity of the first instar males. If such long perdurance of maternal products is frequent, it should be an important consideration when interpreting mutant phenotypes.

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