# Evolutionary genomic change paralleled by differential responses of 2× and 4× calli cultures

U. C. Lavania and S. Srivastava

*Central Institute of Medicinal and Aromatic Plants, P.O. Ram Sagar Misra Nagar, Lucknow-226 016 (India)*
*Received 5 May 1989; accepted 14 September 1989*

*Summary.* A comparative account of numerical chromosome variation in 2× and 4× callus cultures of *Hyoscyamus muticus* examined at monthly intervals reveals that both types of calli tend to attain a genomic flux around 4×. Parallels apparently exist between the course of ploidy change and chromosomal flow in culture and evolutionary processes in nature, suggesting the utility of the in vitro system as a rapid approach for recognizing trends in genome evolution.
*Key words.* Callus cytology; chromosomal flow; genome evolution; polyploid callus; *Hyoscyamus muticus.*

The detection of somatic polyploidy as being the rule rather than the exception in many animals and plants[1], and in transposable elements in maize[2], has led to the development of new ideas about genome variability and fluidity. Plants show far more changes at the DNA and chromosome levels than animals[3]. Such changes are even more evident when tissues or cells are removed from the plant and cultured on artificial media, when they frequently give rise to somatic cells showing a high incidence of both gross and subtle genetic changes[4,5]. The changed DNA sequences and chromosomes can pass through meiosis and contribute to evolution[3,6]. Since in an in vitro system such changes can be studied much more rapidly than in an in vivo one, the relative flow of chromosome variation was explored in callus cultures of two ploidy levels, with a view to simulating evolutionary divergence.

## *Materials and methods*

Callus cultures were developed from stem explants of germinating seedlings of diploid *Hyoscyamus muticus* (2n = 2× = 28) and its stable autotetraploid (4× = 56, strain CIMAP/HMT-1)[7,8]: Both 2× and 4× calli were regularly subcultured on fresh medium[8] at monthly intervals and concurrently examined for somatic chromosome number variation, using the aceto-orcein squash technique. The data (table) on chromosome variation were recorded on a total of approximately 100–150 well-analyzable cells in each case.

## *Results*

Both 2× and 4× calli in the initial stages of subculture exhibited primarily the chromosomal mode of the starting material, but subsequently acquired a heterogenous status, with cells with chromosome numbers varying from the 2× to the 8× level. However, a genomic level of 4× was preponderant in the cell population. With the passage of time the 2× callus culture tended to attain the 4× genomic level whereas the 4× callus culture tended to maintain it. Nevertheless, the frequency of 4× cells went on gradually decreasing in the 4× callus culture, until after 8–9 months of subculture a genomic equilibrium was established similar to that in the 2× callus derived cell population. The observations on the frequency of cells with 4× chromosome level in cell populations derived from 2× and 4× callus are depicted in figure 1, to illustrate the relative chromosome flow in the callus cultures of the two ploidy levels during the process of repeated subculturing.

Trends in numerical chromosome variation in callus cultures of diploid and autotetraploid *Hyoscyamus muticus* over a period of one year at monthly subculture intervals

| Passage of subculture (no. of months) | Frequency of cells (%) in particular chromosome variant class | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Diploid callus | | | | | Tetraploid callus | | | | |
| | 2× (22–35) | 3× (36–49) | 4× (50–63) | 4×–8× (64–112) | 8× (>112) | 2× (22–35) | 3× (36–49) | 4× (50–63) | 4×–8× (64–112) | 8× (>112) |
| 1 | 69.2 | 28.9 | 1.9 | – | – | 13.1 | 27.4 | 54.1 | 5.4 | – |
| 2 | 62.8 | 30.2 | 4.7 | 2.3 | – | 6.7 | 21.3 | 57.3 | 14.7 | – |
| 3 | 42.9 | 35.7 | 16.1 | 3.5 | 1.8 | 6.0 | 29.1 | 50.5 | 14.4 | – |
| 4 | 51.0 | 33.8 | 8.4 | 5.1 | 1.7 | 6.7 | 24.4 | 48.7 | 20.2 | – |
| 5 | 40.7 | 27.7 | 18.7 | 12.9 | – | 7.1 | 26.1 | 48.1 | 17.2 | 1.5 |
| 6 | 36.9 | 34.8 | 26.1 | 2.2 | – | 10.6 | 15.6 | 44.8 | 27.3 | 1.7 |
| 7 | 33.1 | 25.5 | 35.2 | 4.3 | 1.9 | 2.9 | 34.9 | 44.3 | 17.9 | – |
| 8 | 33.1 | 29.8 | 30.1 | 7.0 | – | 5.1 | 35.5 | 37.1 | 19.8 | 2.5 |
| 9 | 26.0 | 29.9 | 28.2 | 9.9 | 6.0 | 6.1 | 24.0 | 36.1 | 28.6 | 5.2 |
| 10 | 16.6 | 33.3 | 40.2 | 4.9 | 5.0 | 7.0 | 24.1 | 32.0 | 29.6 | 7.3 |
| 11 | 15.0 | 31.7 | 43.3 | 6.7 | 3.3 | 6.5 | 31.2 | 33.5 | 20.1 | 8.9 |
| 12 | 20.0 | 31.4 | 42.9 | 2.9 | 2.8 | 9.1 | 22.3 | 33.2 | 25.3 | 10.1 |

Numbers in parentheses indicate range in chromosome number; 2× = 28 ± 7; 3× = 42 ± 7; 4× = 56 ± 7; 2n = 28.

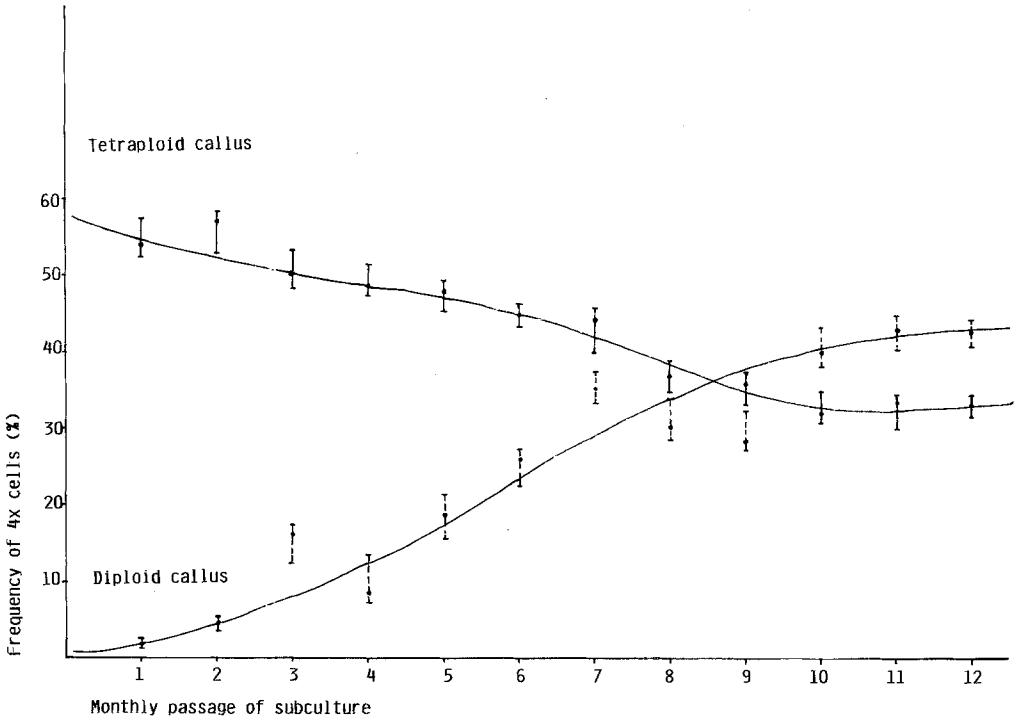


Figure 1. Relative sequential flow of 4× cells in diploid- and autotetraploid-derived callus cultures of *Hyoscyamus muticus*.

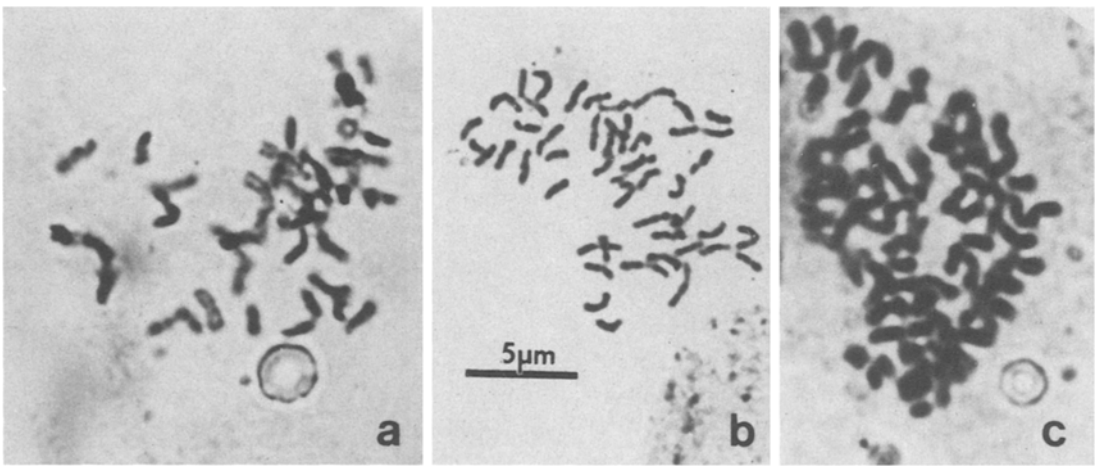


Figure 2. Callus cytology of *Hyoscyamus muticus* (2× = 28) showing numerical chromosome variation: *a* 42 chromosomes; *b* 50 chromosomes; *c* 63 chromosomes.

*Discussion*

Most phylogenetic changes in the genome are brought about by the same mechanism as ontogenetic changes, i.e. polyploidization, DNA amplification and elimination, mutation and rearrangement, followed by fixation of the new genomic and karyotypic state. Of these, chromosomal structural alterations coupled with polyploidization are major contributors to evolution and divergence, at least in plants. Because genomic changes and their fixation occur rapidly in cell and tissue cultures, these techniques could serve as useful models for evolutionary events. Culture conditions which might be stressful for the cells may reflect a changing environment in nature [3]. The mechanisms that generate rapid genomic changes can be envisaged as a metabolic feedback process which may induce adaptation, or as the response to genetic stress [9, 10].

Numerous reports available in the literature dealing with a wide range of species reveal that a tremendous amount of variation in chromosome number and structure can

arise as a consequence of callus and suspension culture[11]. The occurrence of all sorts of chromosomal variations, including numerical, ploidy, structural and also polytenic ones has been elegantly demonstrated in suspension cultures of wheat[5]. All such changes have been widely documented in the literature to account for the chromosomal basis of speciation. It was not possible to precisely study structural changes in the chromosomes in the present study on *Hyoscyamus* callus, owing to the very small size of its somatic chromosomes[12] (fig. 2). The present information is relevant for genome evolution via polyploidization – one of the major factors considered to be responsible for divergence and speciation in plants. The predominant occurrence of tetraploid cells in both 2× and 4× calli cultured over a period of time indicates that the 2× acquires the genomic dosage of 4× as a sequel to the stress enforced during artificial culture. Since this ampflified genome dosage appears to help to overcome the stress of culture, it seems reasonable to suppose that it is in order to overcome stress that the evolution of plants has taken place primarily in the forward direction by elevation of the ploidy level and enhancement of chromosome number. This theory is also consistent with the observation that polyploids are less vulnerable to induced mutagenic variation or evolutionary change compared to diploids. The present comparative study of 2× and 4× genotypes suggests that the 4× genotype is less constrained in artificial cultures, as genome multiplication brings about a buffering action. The artificial culture conditions could be assumed to resemble in some ways the stressful environment which enforces speciation and adaptation in nature.

Acknowledgments. We are grateful to our colleagues Dr P. S. Ahuja and Mr C. C. Giri for rendering their valuable help in in vitro culture techniques. Our thanks are also due to the Director, CIMAP for facilities provided and CSIR, New Delhi for financial assistance to S. S. (CIMAP publication no. 17/89).

1 Nagl, W., Endopolyploidy and Polyteny in Differentiation and Evolution. Elsevier/North Holland, Amsterdam 1978.
2 McClintock, B., Cold Spring Harbor Symp. Quant. Biol. *16* (1951) 13.
3 Nagl, W., Biotechnology in Agriculture and Forestry, Vol. 11. Ed. Y. P. S. Bajaj. Springer, Heidelberg/New York 1989.
4 Orton, T. J., Iowa State J. Res. *61* (1987) 481.
5 Karp, A., Wu, Q. S., Steele, H. S., and Jones, M. G. K., Theor. appl. Genet. *74* (1987) 140.
6 Buiatti, M., Plant Cell, Tissue, and Organ Culture, p. 358. Eds J. Reinert and Y. P. S. Bajaj. Springer, Berlin 1977.
7 Lavania, U. C., Theor. appl. Genet. *73* (1986) 292.
8 Lavania, U. C., and Srivastava, S., Protoplasma *145* (1988) 55.
9 Walbot, V., and Cullis, C. A., A. Rev. Plant Physiol. *36* (1985) 367.
10 McClintock, B., Science *226* (1984) 792.
11 Bayliss, M. W., Int. Rev. Cytol. (Suppl.) *11A* (1980) 113.
12 Srivastava, S., and Lavania, U. C., Nucleus *30* (1987) 94.

0014-4754/90/030322-03$1.50 + 0.20/0


## Sylvaticin: A new cytotoxic and insecticidal acetogenin from *Rollinia sylvatica* (Annonaceae)

K. J. Mikolajczak[a], R. V. Madrigal[a], J. K. Rupprecht, Y.-H. Hui, Y.-M. Liu, D. L. Smith and J. L. McLaughlin*

[a] *USDA-ARS, Northern Regional Research Center, 1815 N. University St., Peoria (Illinois 61604, USA), and Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette (Indiana 47907, USA)*
*Received 21 June 1989; accepted 18 August 1989*

*Summary.* Sylvaticin (**I**), a new tetrahydroxy annonaceous acetogenin with nonadjacent tetrahydrofuran rings, has been isolated from the dried fruits of *Rollinia sylvatica* St. Hil. (Annonaceae). This compound is extremely cytotoxic to human tumor cells and shows promising insect control properties.
*Key words.* Sylvaticin; acetogenin; *Rollinia sylvatica*; Annonaceae; brine shrimp; cytotoxicity; insecticidal; striped cucumber beetle; European corn borer.

The tetrahydrofuran acetogenins represent a new group of diversely bioactive (antitumor, cytotoxic, antimicrobial, and antimitotic) natural compounds[1–18]. Our report of the pesticidal activity of the bistetrahydrofuran acetogenin, asimicin, from *Asimina triloba* Dunal. (Annonaceae)[7], further expanded the spectrum of biological activities of this new class of natural compounds. More recently we have reported promising pesticidal activity for the bistetrahydrofuran acetogenin, bullatacin, from *Annona bullata* Rich. (Annonaceae)[15], and lesser pesticidal activity for the monotetrahydrofuran acetogenins, goniothalamicin and annonacin, from *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae)[10]. Also, we have recently patented the pesticidal uses of the acetogenins[7].

The crude hexane extract of *Rollinia sylvatica* St. Hil. (Annonaceae) fruit produced high mortality when fed to European corn borer larvae [*Ostrinia nubilalis* (Hübner)] in an artificial diet and was very toxic to brine shrimp larvae [*Artemia salina* (Leach)]. Fractionation of the extract was guided by the brine shrimp lethality bioassay[19] and by thin-layer chromatographic monitoring. After solvent partitioning and repeated column and thin-layer chromatographic separations, sylvaticin (**I**), one of the