an$_1$, *bi*$_1$; strain D: *bi*$_1$, *meth*$_1$, *fl*$_1$; strain E: *ad*$_{14}$, *y*, *co*; strain F: *su*$_1$*ad*$_{20}$, *paba*$_1$, *y*, *ad*$_{20}$, *Acr*, *lys*$_5$, *cha*. Mutant alleles in this study determined the phenotypes: *y*, yellow conidia; *w*$_2$ (epistatic to *y*/*y*+), white conidia; *cha*, chartreuse conidia; *co*, compact colonies; *fl*$_1$, fluffy mycelium; *ad*$_{14}$ and *ad*$_{20}$, *an*$_1$, *bi*$_1$, *lys*$_5$, *meth*$_1$, *nic*$_2$, *paba*$_1$, *pyro*$_4$, *ribo*$_5$, *s*$_{12}$, requirements, respectively, for adenine, aneurine, biotin, lysine, methionine, nicotinic acid, *p*-aminobenzoic acid, pyridoxine, riboflavine and thiosulfate; *Acr*$_1$, resistance to acriflavine; *su*$_1$*ad*$_{20}$, suppressor of adenine requirement caused by *ad*$_{20}$. Origin and location of the mutants can be found in BARRATT, JOHNSON and OGATA[7] and BALL and AZEVEDO[8]. General techniques were those of PONTECORVO et al[6]. The 6 strains were crossed in all possible combinations (15 crosses). After 2 days incubation the mycelial mat was transferred to dishes of solid MM (plus 0.02 µg/ml biotin for the cross C×D). After 28 days incubation at 37°C a counting of cleistothecia was carried out for each heterokaryon. The number of cleistothecia in 10 fields (7.2 mm diameter each) chosen at random in 4 heterokaryons, for each cross, was scored. For each cross 2 independent counts were performed. Statistical analysis of the data was carried out following the method suggested by GRIFFING[9]. The mathematical model in this is as follows: $x_{ij} = \mu + g_i + g_j + s_{ij} + \bar{e}_{ij}$ in which μ is the general mean, g_i and g_j the effects of the general combining ability, s_{ij} the effect of the specific combining ability so that $s_{ij} = s_{ji}$ and $\bar{e}_{ij}$ is the exponential error from the observations of the order *ij*. The restrictions are $\Sigma g_i = 0$ and $\Sigma s_{ij} = 0$ (for each *j*).

Results and discussion. Table I gives the mean frequency of cleistothecia per mm² for each cross. From these data, the mean squares of the general and specific combining abilities and of the effects of the general combining ability for each cross were estimated (Tables II, III and IV). The application of the method of GRIFFING[9] to fungi seems viable in the case of heterokaryons since they present certain similarities with the diploid state[10]. The method assumes that when a set of inbred lines is used in a diallel crossing system, a genetic interpretation in terms of quantitative inheritance is made possible since the analysis is an analysis of the combining abilities of the gametes. Thus, the general properties of a diploid individual may be regarded as the combination of the genetic properties of the 2 gametes which united to form the individual. Therefore in the statistical analysis the genotypic effect of an individual may be considered as the summation of effects contributed by each gamete (that is, set of genes in the gamete) and the interaction of gametes (that is, interaction of the genes in one gamete with those in the other). Heterokaryons are not products of gamete fusion; they are, in fact, products of genes in each nucleus and, in the statistical analysis, the genotypic effect of a heterokaryon is the summation of effects given by each nucleus and interaction between such nuclei. The observed value of a given cross can be represented linearly by the estimates of combining abilities. The values of the general combining ability are an indication of the importance of the additive genic effects and the values of the specific combining abilities indicate the importance of the genic effects of dominance and epistasis[11]. The results obtained show that the genetic variation expressed by the amount of cleistothecia produced in different crosses is due, not only to the genic additive effects, but also to dominant and possibly epistatic effects (Table II). There is no doubt that dominance is a characteristic of some of the factors involved in the control of the character studied but other factors which do not show dominance are also present. The strains differed in their content of factors that increase the number of cleistothecia (Table III). Table IV gives the relative importance of the effects of the specific combining ability showing that certain pairs of strains have genetic constitutions that can complement each other.

Fungi present many advantages for genetic studies, including rapid growth, the large number of individuals which can be analyzed, and ease of handling and storage. Several characteristics presented by these organisms are suitable for quantitative genetic studies using the same process which was used in the present work. Many of the common industrial fungi readily form heterokaryons and diploids. The production of valuable substances such as antibiotics is directed by quantitative genes[12]. Thus quantitative genetic studies in industrial fungi can play an important role comparable with that achieved by these methods in plant and animal breeding.

Resumen. Cruzamiento dialélico entre seis cepas del hongo filamentoso *Aspergillus nidulans* fué analizado estadísticamente por el método sugerido por GRIFFING[9] visando establecer los valores de la capacidad general de combinacion y de la capacidad específica de combinación para la cantidad de cleistotécios. Los resultados obtenidos parecen indicar la participación de genes que exhiben effectos aditivos y dominantes. Es sugerido que el método puede ser aplicado en el analisis de otras características incluyendo aquellas de valor económico como producción de antibióticos.

I. R. BARACHO and J. L. AZEVEDO

Department of Plants Genetics, University of Campinas, Campinas, São Paulo (Brazil) and Department of Genetics, University of São Paulo, Piracicaba, P.O. Box 83, São Paulo (Brazil),
24 December 1971.

[7] R. W. BARRATT, G. B. JOHNSON and W. N. OGATA, Genetics *52*, 233 (1965).
[8] C. BALL and J. L. AZEVEDO, Aspergillus Newsl. *5*, 9 (1964).
[9] B. GRIFFING, Aust. J. biol. Sci. *9*, 463 (1956).
[10] I. R. BARACHO, R. VENCOVSKY and J. L. AZEVEDO, Trans. Br. mycol. Soc. *54*, 109 (1970).
[11] G. F. SPRAGUE and L. A. TATUM, J. Am. Soc. Agron. *34*, 923 (1942).
[12] G. SERMONTI, *Genetics of Antibiotic Producing Microorganisms* (Interscience, London 1969), p. 389.

Lampbrush Chromosomes from Semi-Albino Crested Newts, *Triturus Cristatus Carnifex* (Laurenti)[1]

Semi-albinism, also known in amphibians as mutation 'yellow', does not affect the process of melanogenesis which takes place during embryonic and larval life, while it produces changes in melanin granules of pigmented cells at metamorphosis[2]. Some data concerning spermatogenesis of semi-albino crested newts were already available[2], whereas nothing was known so far on karyological aspects of oogenesis. For this reason we have carried out, on some of these mutants[3], a study of lampbrush chromosomes which, being 'high resolution chromosomes', had

already been considered to represent a suitable material for identification and exact interpretation of structural rearrangements, however minute, either spontaneous[4] or experimentally induced[5]. After having preliminarly controlled number and general morphology of bivalents, isolated from several oocytes of 3 mutant females, we performed a close investigation into the morphological details of the main chromosome features (giant loops and other single landmarks) since semi-albinism was known to be due to a gene mutation. This study has therefore led us to the comparison of the morphology of lampbrush chromosomes isolated from semi-albino females with the chromosome maps already available for *T. c. carnifex*[6], which allow the 12 bivalents of the haploid set of the species to be identified and characterized. Moreover, we compared the morphology of the main loops and landmarks from semi-albinos with that of the same loops and landmarks inserted on lampbrush chromosomes prepared as a control from wild type females, collected in different Italian localities such as Turin, Pisa and Naples. For both comparisons, a number of oocytes of different size, excised from ovaries at various stages of the seasonal cycle, have been used. Manipulation of nuclei and isolation of chromosomes have been accomplished according to technique previously described[7].

The results can be summarized as follows. Neither genome nor chromosome mutations have been detected in lampbrush karyotypes confirming the observations already

[1] This work was financially supported by C.N.R., Rome.

[2] E. Capanna, Boll. Zool. *34*, 100 (1967).

[3] We thank Prof. E. Capanna for having kindly supplied us with the semi-albino specimens used in the present work.

[4] G. Mancino, I. Nardi and G. Barsacchi, Cytogenetics *9*, 260 (1970).

[5] J. C. Lacroix and M. T. Loones, Chromosoma *36*, 112 (1971).

[6] H. G. Callan and L. Lloyd, Phil. Trans. R. Soc. B *243*, 135 (1960).

[7] J. G. Gall, in *Methods in Cell Physiology* (Ed. D. Prescott; Academic Press Inc., New York 1965), p. 37.

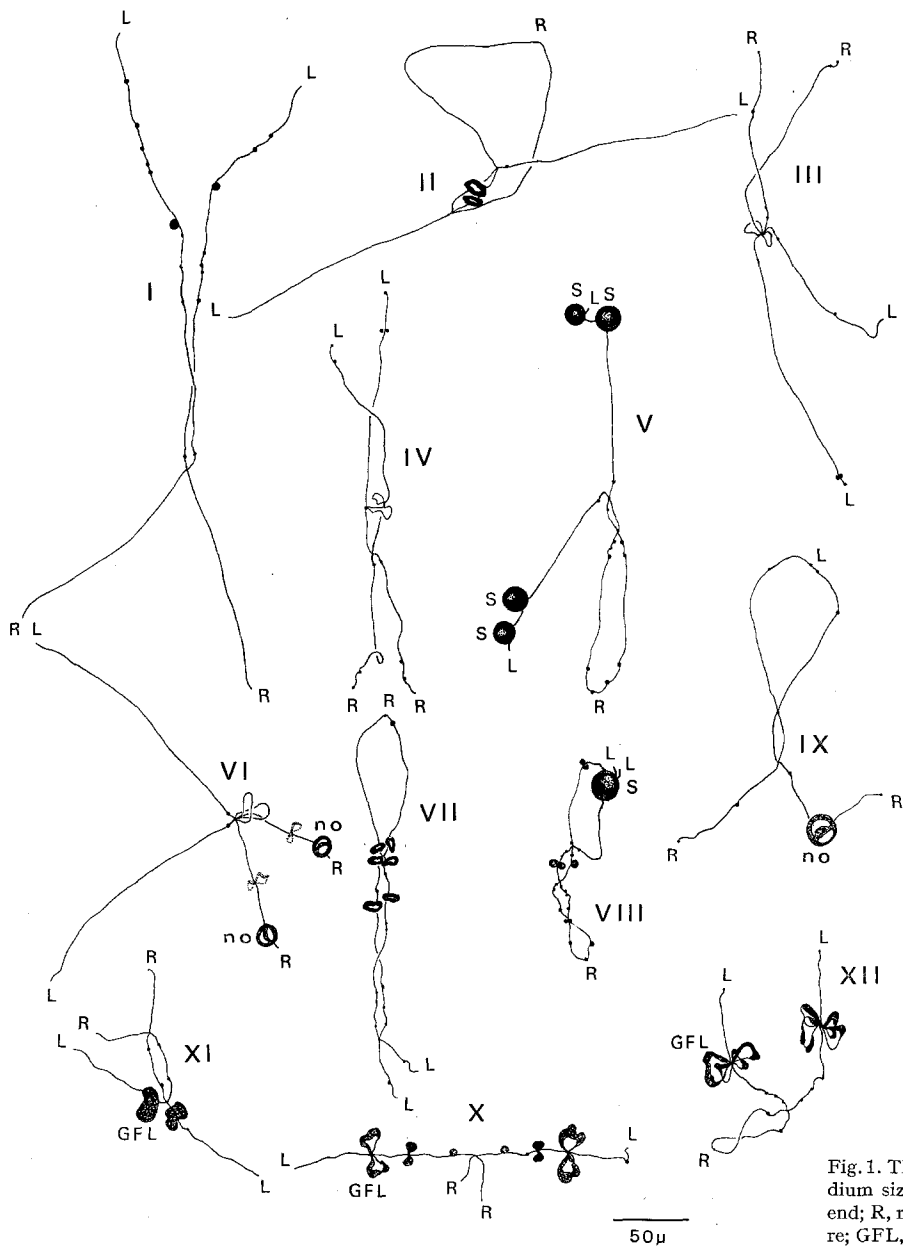

Fig. 1. The 12 lampbrush chromosomes from a medium size oocyte of a semi-albino mutant. L, left end; R, right end; no, nucleolar organizer; S, sphere; GFL, giant fusing loop.

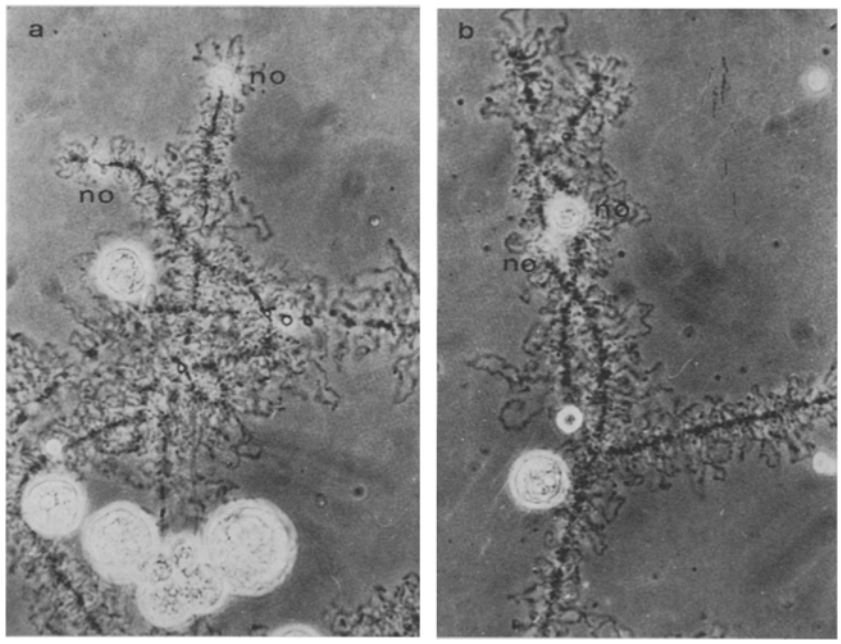

Fig. 2. Microphotographs of the nucleolar organizers (no) inserted on chromosome VI (a) and on chromosome IX (b). ×545.

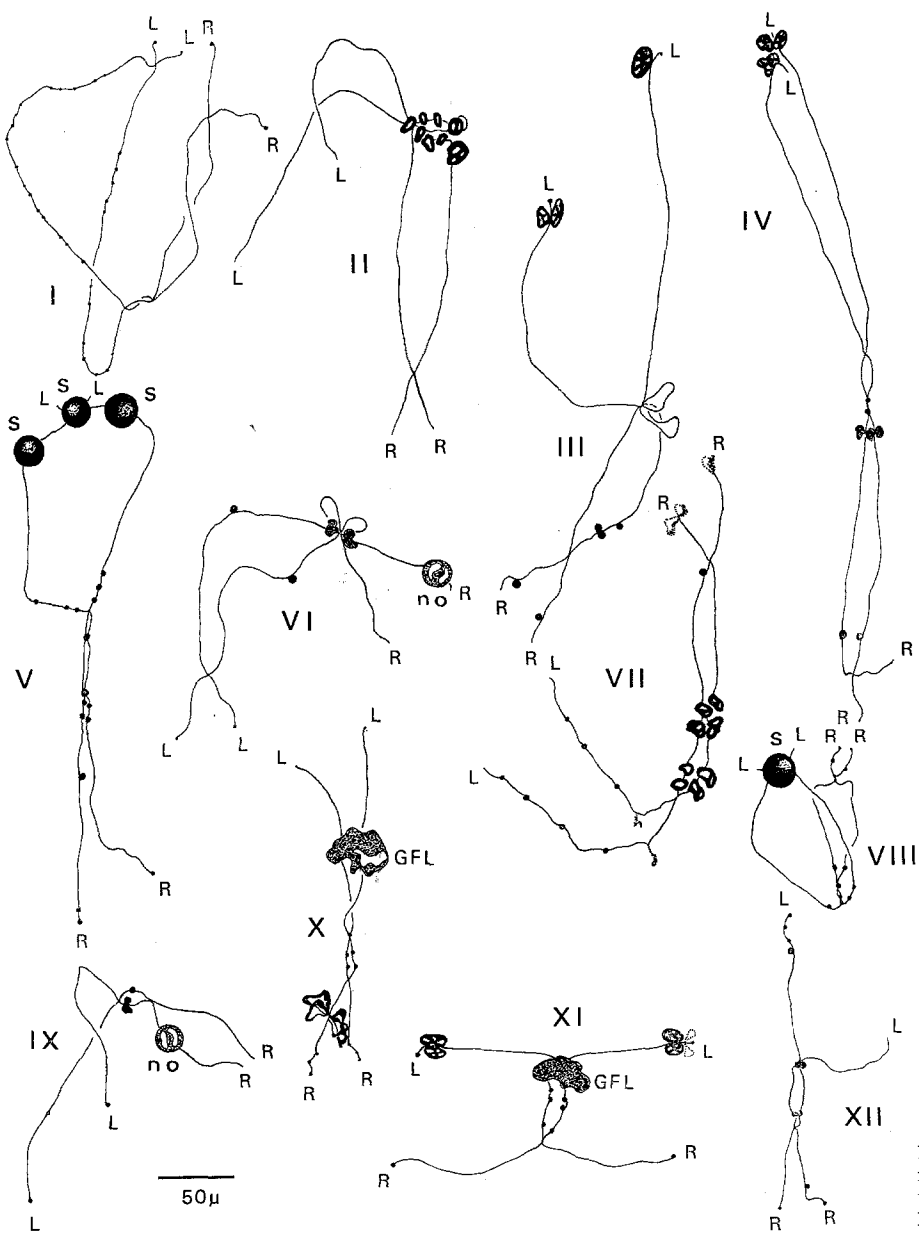

Fig. 3. The 12 lampbrush chromosomes from a medium size oocyte (of a wild type female) collected near Pisa. Same lettres as in Figure 1.

made on spermatogonia and spermatocytes I[2]. In addition, the fine comparison of the characters of each bivalent with the correspondent map has not revealed any particular modification of the morphology of single giant loops and other landmarks which could be related to semi-albinism. As regards these results, it must be pointed out that such analysis, however detailed, is hindered by the different physiological conditions of oocytes which modify to some extent the morphology, the texture and the dimensions of the loops and other lateral structures[8,9], such conditions being related to the stage of growth and the rate of endogenous syntheses. Moreover, the discovery of gene mutations throughout the analysis of the morphology or 'phenotype' of the lampbrush loops is at present very limited in European newts, owing to the lack of a sufficient knowledge of formal genetics[10], as well as the difficulty in establishing the relationship between the single structures identifiable along the lampbrush chromosomes and the single genetically defined units of inheritance.

In the course of this investigation on lampbrush chromosomes of semi-albinos, we have identified the nucleolar organizers which were not previously recognized in *T. c. carnifex*[6,8,9]. In most oocytes a nucleolus is inserted on chromosomes VI and IX in left subterminal region (Figures 1 and 2); it shows varying morphological conditions, as was pointed out also for *T. viridescens*[11] and *T. marmoratus*[12]. A similar observation has been made on lampbrush chromosomes from wild type females, whose chromosomes VI and IX often bear a nucleolus at the same chromosome sites (Figure 3). The localization of the nucleolus organizing regions, calculated on several oocytes of both semi-albino and dark females and expressed in units (1 units = 1/100 of the length of chromosome XII whose length is made equal to 100 units[13]) is 195 units for chromosome VI and 122 units for chromosome IX. The nucleolar organizers therefore show a localization different from that of the giant fusing loops inserted on chromosomes X, XI and XII, which were interpreted as possible sites of production of nucleolar material[14]. These findings account for the completion of the chromosome maps of *T. c. carnifex* which has been made in the present study (Figure 4), since complete maps are requested for a more exact cytotaxonomic and cytogenetic comparison between *T. cristatus* and the closely related species *T. mar-*

[8] H. G. Callan, in *International Review of Cytology* (Eds. G. H. Bourne and J. F. Danielli); Academic Press Inc., New York 1963), p. 1.
[9] H. C. Macgregor, Q. Jl microsc. Sci. *104*, 351 (1963).
[10] H. Spurway, in Symp. Soc. exp. Biol. *7*, 200 (1953).
[11] J. G. Gall, J. Morph. *94*, 283 (1954).
[12] I. Nardi, M. Ragghianti and G. Mancino, Chromosoma, *37*, 1 (1972).
[13] G. Mancino and G. Barsacchi, Caryologia *18*, 637 (1965).
[14] H. C. Macgregor, Q. Jl. microsc. Sci. *106*, 215 (1965).

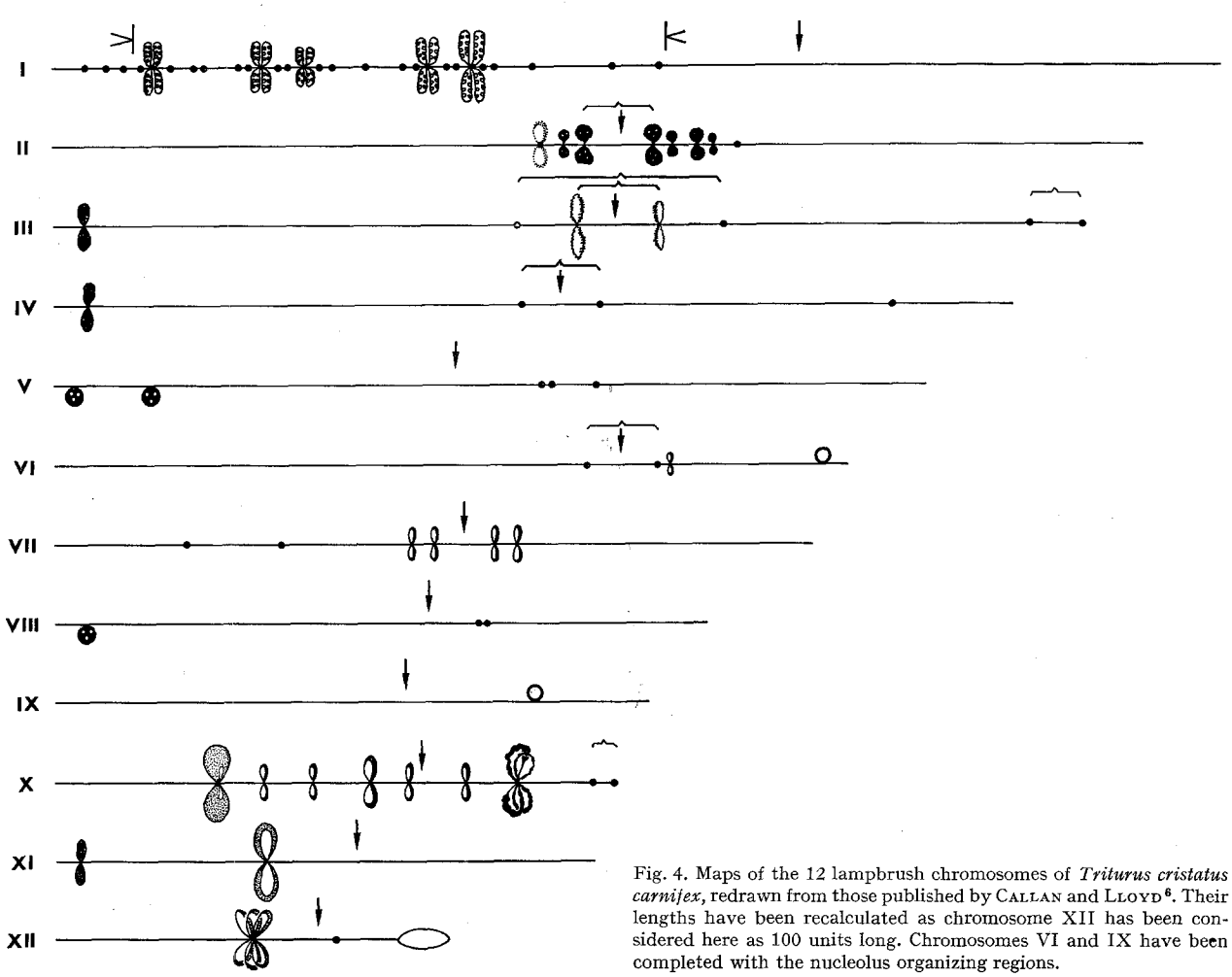

Fig. 4. Maps of the 12 lampbrush chromosomes of *Triturus cristatus carnifex*, redrawn from those published by Callan and Lloyd[6]. Their lengths have been recalculated as chromosome XII has been considered here as 100 units long. Chromosomes VI and IX have been completed with the nucleolus organizing regions.

moratus, whose mitotic and lampbrush karyotypes have been recently defined[12,15,16].

Riassunto. Sono stati studiati i «lampbrush chromosomes» di mutanti semi-albini di *Triturus cristatus carnifex*. Il rilevamento degli organizzatori nucleolari ha permesso il completamento delle mappe cromosomiche della specie.

G. MANCINO, IRMA NARDI and MATILDE RAGGHIANTI

Cattedra di Istologia e Embriologia, Istituto di Zoologia e Anatomia Comparata dell'Università, Via Volta 4, Pisa (Italy), 7 February 1972.

[15] I. NARDI and G. MANCINO, Experientia *27*, 424 (1971).
[16] G. MANCINO and I. NARDI, Experientia *27*, 821 (1971).

Der Mitosezyklus im diploiden und triploiden Wurzelmeristem von *Scilla sibirica*

Die Dauer des Mitosezyklus und seiner Abschnitte im meristematischen Gewebe der Wurzelspitze ist bei einigen Pflanzenarten, die von cytologischem Interesse sind, bestimmt worden (z.B. *Tradescantia paludosa*[1], *Vicia faba*[2], *Trillium grandiflorum*[3], *Haplopappus gracilis*[4], *Allium cepa*[5] und *Trillium erectum*[6]). *Scillia sibirica*, dem Cytologen bekannt vor allem durch die Untersuchungen von LACOUR und RUTISHAUSER[7] über die Existenz von Subchromatiden, verdient besondere Aufmerksamkeit, weil diese Spezies die Vorteile grosser, in der Regel unterscheidbarer Chromosomen und markanter Chromozentren aufweist. Kürzlich ist gezeigt worden, dass auch hier, wie bei vielen anderen Objekten, die DNS des Heterochromatins am Ende der S-Phase synthetisiert wird. Die spätreplizierenden chromosomalen Segmente sind identisch mit den Kältezonen, die bei dieser Art durch extreme Kältebehandlung induziert werden können[8].

Bekanntlich bestehen keine Unterschiede in der DNS-Synthesedauer zwischen diploiden Pflanzen und ihren autotetraploiden Formen[9,10]. Aufgabe dieser Mitteilung ist es, über den Mitosezyklus von *Scilla sibirica* ($2n$) zu berichten und gleichzeitig darzulegen, ob die Anwesenheit eines zusätzlichen Chromosomensatzes ($3n$) zu einer Veränderung des Zyklus und seiner einzelnen Phasen führt.

Material und Methoden. Als Versuchsobjekt dienten diploide ($2n = 12$) und triploide ($3n = 18$) Zwiebeln («Spring Beauty»), die im Handel lediglich während des Herbstes im nicht ausgetriebenen Zustand erhältlich sind. Die damit verbundene Abhängigkeit der Versuchsplanung von der Jahreszeit kann grossenteils verhindert werden, indem man die Zwiebeln eine Viertelstunde lang in einer 0,2 prozentigen 8-Hydroxychinolinsulfat-Lösung desinfiziert und anschliessend trocken in sterilem Sägemehl bei 0 °C aufbewahrt. So ist eine Lagerung während knapp eines Jahres möglich. Die Anzucht der Wurzeln geschah wie bereits früher berichtet[8].

Nach einer zweistündigen Fütterung der Pflanzen mit methylmarkiertem ^{3}H-TdR (2 µCi/ml, 18600 mCi/m*M*, Radiochemical Centre, Amersham) wurden in Abständen von zwei und mehr Stunden Wurzeln abgeschnitten und direkt in Methanol/Eisessig (3:1) während 30 min fixiert. Zwecks Überprüfung der chromosomalen Markierungsmuster geschah die Fixation bei einigen Erholungszeiten zusätzlich erst nach Vorbehandlung mit Colchicin (0,05% in Leitungswasser, 2 h). Die weitere Verarbeitung der Wurzeln erfolgte mit Hilfe der Feulgen-Quetschmethode und anschliessender Autoradiographie (Stripping Film, Kodak AR 10). Für das cytologische Präparat wurde jeweils nur eine etwa 0,5 mm lange, stark gefärbte Meristemzone gequetscht, nachdem die Wurzelhaube an Filterpapier abgestreift worden war. Für die Bestimmung des Mitosezyklus wurden einerseits zwei Wurzeln einer diploiden Pflanze pro Erholungszeit verwendet, während an-

[1] D. E. WIMBER, Am. J. Bot. *47*, 828 (1960).
[2] H. J. EVANS and D. SCOTT, Genetics *49*, 17 (1964).
[3] C. J. GRANT, Mutation Res. *2*, 247 (1965).
[4] E. SPARVOL, H. GAY and B. P. KAUFMANN, Caryologia *19*, 65 (1966).
[5] R. MATAGNE, Caryologia *21*, 209 (1968).
[6] E. R. BOOTHROYD and N. MACDONALD MARK, Can. J. Genet. Cytol. *12*, 750 (1970).
[7] L. F. LACOUR and A. RUTISHAUSER, Chromosoma *6*, 696 (1954).
[8] T. W. BAUMANN, Expl Cell Research *64*, 323 (1971).
[9] M. R. TROY and D. E. WIMBER, Expl Cell Research *53*, 145 (1968).
[10] D.-P. YANG and E. O. DODSON, Chromosoma *31*, 309 (1970).

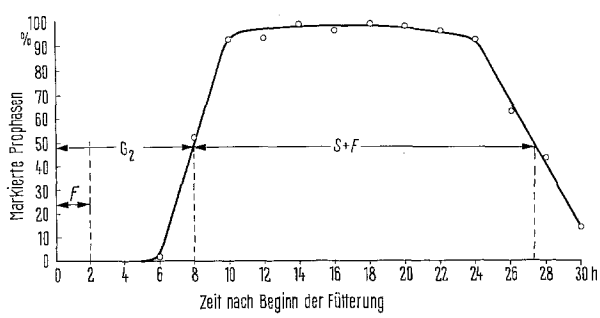

Fig. 1. Markierungsindex-Zeit-Diagramm, *Scilla sibirica* $2n = 12$. Temperatur 23°C. *F*, Fütterungszeit; G_2, postsynthetische Interphase; S, DNS-Synthesephase.

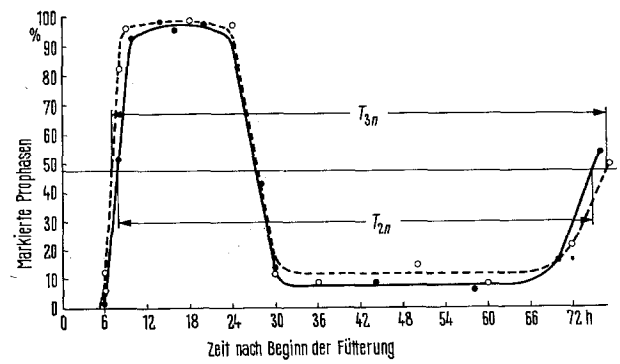

Fig. 2. Mitosezyklus und Ploidiegrad. T_{2n} bezeichnet die Zyklusdauer im diploiden, T_{3n} diejenige im triploiden Meristem. Temp. 23°C.