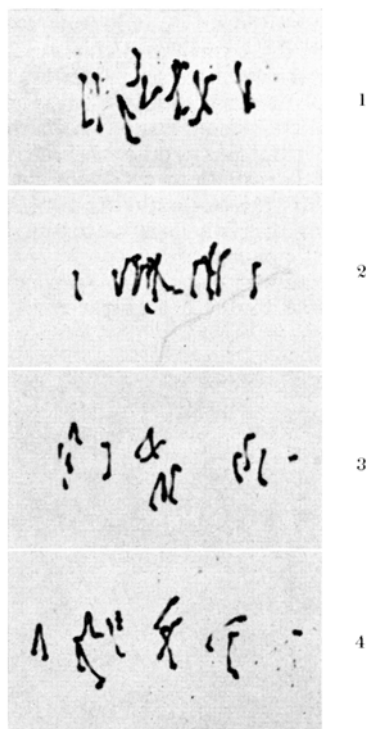


No other coccinellid is known with a chromosome number in excess of $2n = 18 + X\gamma$, although several have lower numbers³. In view of (1) the presence of large



Figs. 1–4.—First meiotic metaphases in 2 males of *Chilocorus stigma*.

Figs. 1 and 2.— $9_{II} + 2_{III}$ (A and B) + X.

Fig. 3.— 7_{II} (2 at right heteromorphic) + 3_{III} (from left to right B, A, and C) + X.

Fig. 4.— 7_{II} (2 near centre heteromorphic) + 3_{III} (from left to right A, B, and C) + X.

Feulgen-light green squash preparations ca. $\times 750$.

masses of heterochromatin visible in *C. stigma* at pachytene; (2) the high frequency at diplotene of ring bivalents that resolve into simple rods by metaphase; and (3) the fact that whole chromosome arms or parts thereof can be lost with impunity in the formation of "fusion" products and heteromorphic bivalents, respectively, it seems reasonably certain that a process of centromere misdivision, of the type known in the plant *Campanula*⁴, followed by the accretion of heterochromatin in the acquisition of new arms must have been the means by which the putative $2n = 27/28$ prototype evolved. If this is so and since the basic chromosome formula of the Coccinellidae is most probably $18 = X\gamma$ ⁵, it appears likely that individuals with 4 trivalents exist, thus extending the theoretical limit of the species downwards to 19 chromosomes. Furthermore, since the chromosomes of this species evidently have a strong predisposition to "fuse", the possibility is open for matings between individuals carrying different combinations of "fused" chromosomes to have given rise to ring complexes analogous to, but structurally different from, those known in several plant genera.

It is, of course, not inconceivable that a similar situation exist in *Gryllotalpa gryllotalpa* and that its baffling numerical differences, which WHITE² was loath

to attribute to "centric fusion" because of the meta-centric nature of the chromosomes, might in fact also have arisen by dispensing with genetically inert arms at the time of "centric fusion".

Postscript. Since this note was sent to press, material from stock cultures of Californian origin, kindly donated by JOHN J. DREA, Department of Biological Control, University of California, Albany, California, has proved the existence of one of the expected homozygous fusion types in the form of males with the lower diploid chromosome number of 22. At meiosis these are present as two ring bivalents, both homozygous for a centric fusion, and nine rod-shaped bivalents. Of particular interest is the absence of an independent X-chromosome in these stocks: it is fused with one of a pair of autosomes, thus forming a heteromorphic rod-bivalent and constituting a new, neo-XY, sex-determining mechanism (AX:A). This discovery of polymorphism among the sex chromosomes increases the potential number of chromosomally different types of both sexes from 2×3^5 to 5×3^5 , or 1215, and further extends the range of diploid numbers downwards to a theoretical 18.

I shall be glad to correspond with people who anticipate being able to help extend the geographical range of future studies by supplying living males of this and related species.

S. G. SMITH

Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada, November 17, 1955.

Zusammenfassung

Von drei ♂♂ von *Chilocorus stigma* wies ein Tier eine (A), eines zwei (A, B) und das dritte drei (A, B, C) zentrische Fusionen auf; $2n$ war 26, 25 und 24. Beim ♂ mit $2n = 24$ sind mindestens zwei Bivalente heterozygot. Abgesehen vom Unterschied der Geschlechtschromosomen (XX♀:XO♂) sind daher 243 verschiedene chromosomale Typen möglich. $2n$ wird von 21 (♂) zu 28 (♀) variieren. Weitere Fusionen bei den Chromosomen der heterozygoten Paare würden zu $2n = 19$ führen, während sich nach andern Fusions-Kombinationen Ringkomplexe bilden müssten.

La question des hétérochromosomes chez les Sauropsidés. I. Reptiles

En 1934, OGUMA¹, ayant constaté que le mâle de *Lacerta vivipara* possède 36 chromosomes et la femelle 35, admit l'existence d'une digamétie Z-O chez les Reptiles. En 1937, il² retrouve le même type chez une Tortue, *Amyda japonica* (♂: $2N = 64$; ♀: $2N = 63$). MAKINO et ASANA³ étudient deux Agamides avec les résultats suivants: *Calotes versicolor*: ♂: $2N = 12M + 22m$; ♀: $2N = 12M + 21m$. *Sitana ponticeriana*: ♂: $2N = 24M + 22m$; ♀: $2N = 24M + 21m$. (M = macrochromosomes, m = microchromosomes.) NAKAMURA⁴ confirme l'existence d'un Z-O chez la femelle d'une Tortue, *Caretta caretta*, et, chez une autre Tortue, *Chelonia japonica*, MAKINO⁵ compte 56 éléments chez le ♂, 55 chez la ♀. Et, dans le même travail, MAKINO note qu'il a réexaminé les préparations d'OGUMA et qu'il est d'accord avec les conclusions de cet auteur.

D'autre part, MATTHEY⁶ ne trouve aucune différence dans l'équipement chromosomique de *Chamaeleon vulgaris* qui, dans les deux sexes, possède $12M + 12m$. MARGOT⁷ analyse les cinèses d'*Anguis fragilis* et de *Lacerta vivipara*: il y a $20M + 24m$ chez l'Orvet, 36 chromosomes chez le Lézard et il est impossible de mettre en

¹ K. OGUMA, Arch. Biol. 45, 27 (1934).

² K. OGUMA, J. Genet. 34, 247 (1937).

³ S. MAKINO et J. ASANA, Chromosoma 3, 208 (1948).

⁴ K. NAKAMURA, Kromosomo, 5-6, 205 (1949).

⁵ S. MAKINO, Annot. zool. Japon. 25, 250 (1952).

⁶ R. MATTHEY et J. KLAUS, Arch. 18, 1 (1943).

⁷ A. MARGOT, Rev. suisse Zool. 28, 555 (1946).

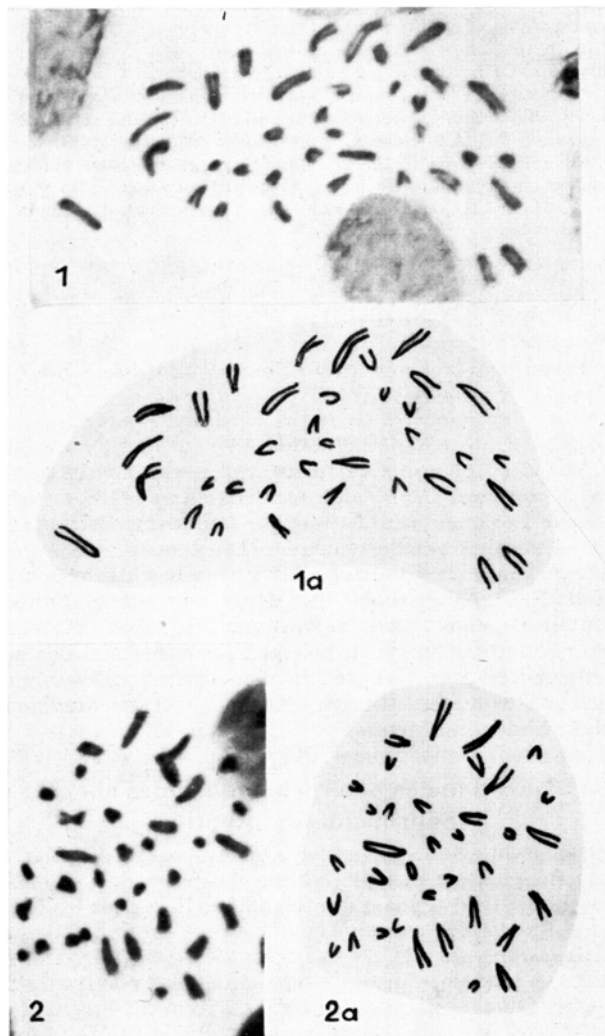
³ S. G. SMITH, Heredity 7, 31 (1953).

⁴ C. D. DARLINGTON and L. F. LA COUR, Heredity 4, 217 (1950).

⁵ S. G. SMITH, Canad. Entom. 82, 58 (1950).

évidence une différence entre les deux sexes. MATTHEY⁸ attache une signification particulière au cas du Caméléon, parce que, chez ce Saurien, les conditions chromosomiques sont si simples que toute erreur d'interprétation est exclue: il considère au contraire que lorsque les chromosomes sont très nombreux et que beaucoup d'entre eux sont très petits, l'évidence objective est in-

beaucoup plus aisée, non que la fixation soit meilleure, mais parce que les figures sont beaucoup plus étalées. Nous avons alors repris le problème, nous attachant essentiellement: a) à élucider le cas de *Lacerta vivipara*; b) à découvrir un Reptile dont l'étude soit plus facile encore que celle du Caméléon vulgaire, de telle sorte qu'un lecteur puisse lui-même se former une opinion en examinant des microphotographies non retouchées. Ajoutons encore ceci: il est toujours difficile de trouver chez la femelle des mitoses nombreuses: or, J. VAN BRINK a découvert que la rate, dans certaines conditions, livre en abondance des cinèses de qualité tout à fait comparable à celle des divisions spermatogoniales du mâle. Après avoir étudié plusieurs espèces de Caméléons, nous avons enfin découvert que le *Chamaeleon bitaeniatus* Fisch. répondait à toutes nos exigences.



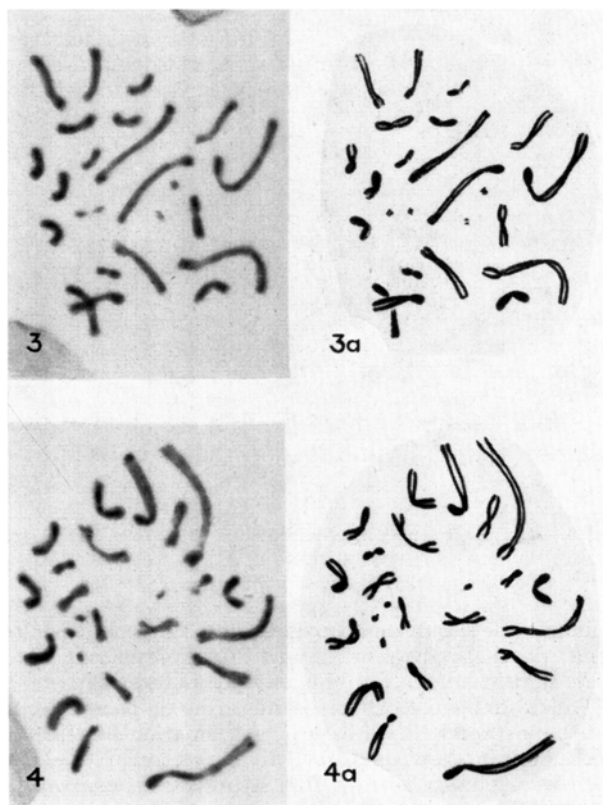
Lacerta vivipara. Préparations obtenues par écrasement. FEULGEN. $\times 1600$.

Fig. 1. Microphotographie d'une mitose chez le ♂.
Fig. 1a. La même: dessin.

Fig. 2. Microphotographie d'une mitose chez la ♀.
Fig. 2a. La même: dessin.

suffisante et que le rôle de l'interprétation personnelle devient excessif.

Tous les travaux que je viens de mentionner sont fondés sur des coupes obtenues à partir d'un matériel fixé par des liquides osmio-chromiques. Or, en ce qui concerne plus spécialement la numération des chromosomes, la technique des «squashes» prétraités à l'eau (MAKINO et NISHIMURA⁹, MATTHEY¹⁰) rend la besogne



Chamaeleon bitaeniatus. Préparations obtenues par écrasement. FEULGEN. $\times 1600$.

Fig. 3. Microphotographie d'une mitose chez le ♂.
Fig. 3a. La même: dessin.

Fig. 4. Microphotographie d'une mitose chez la ♀.
Fig. 4a. La même: dessin.

Lacerta vivipara: les Figures 1 et 1a, 2 et 2a montrent des cinèses empruntées, l'une au mâle, l'autre à la femelle: il est aisé de compter 36 chromosomes dans les deux cas. *Chamaeleon bitaeniatus*: les Figures 3 et 3a, 4 et 4a (♂ et ♀) permettent de dénombrer aisément les chromosomes et d'aboutir à la formule correcte, commune aux deux sexes: $2N = 20M + 4m$. Le petit nombre de microchromosomes autorise, puisque le décompte des grands éléments n'offre aucune difficulté, une conclusion absolument certaine et que nous estimons valable pour l'ensemble des Sauriens: il n'existe pas, dans ce groupe, de chromosomes sexuels à l'échelle morphologique.

⁸ R. MATTHEY *Les chromosomes des Vertébrés* (F. Rouge, Lausanne 1949).

⁹ S. MAKINO et I. NISHIMURA, *Stain Techn.* 27, 1 (1952).

¹⁰ R. MATTHEY, *Rev. suisse Zool.* 60, 225 (1953).

N'ayant pas étudié de Chéloniens, il nous est évidemment impossible de nier *a priori* les affirmations des cytologistes japonais. Cependant, ceux-ci ayant commis une erreur dans le cas du Lézard vivipare, matériel relativement facile, il n'est pas exclu que chez les Tortues, dont le type cinétique est beaucoup plus complexe, ils se soient trompés dans le compte des microchromosomes. Une discussion à ce sujet trouvera place dans la seconde partie de nos recherches, consacrée aux Oiseaux.

R. MATTHEY et J. M. VAN BRINK

Laboratoire de zoologie, Université de Lausanne et Institut de génétique, Université d'Utrecht, le 27 novembre 1955.

Summary

In contradiction to some Japanese authors (OGUMA, MAKINO, NAKAMURA), we are unable to find any difference in the chromosome sets of the male and female of *Lacerta vivipara* ($2N = 36$). In a saurian, where the analysis is very easy (*Chamaeleon bitaeniatus*), there are, without the smallest doubt, 24 chromosomes in both sexes ($20M + 4m$). The evidence of a female heterogamety in turtles is by no means satisfying.

The Mitochondrial Pattern in the Development of the Ascidian Egg

(1)–Janus green has been, for a long time, considered a vital stain specific for the mitochondria. Recent investigations seem to have produced plausible evidence to explain such a specificity: the mitochondria are indeed supposed to be the seat of many enzymes, particularly the cytochrome-oxidase, with which Janus green chemically reacts.

As Janus green in very diluted solutions is not toxic for the cell, it can be very profitably used to follow the modification and distribution of the mitochondria in the developing egg.

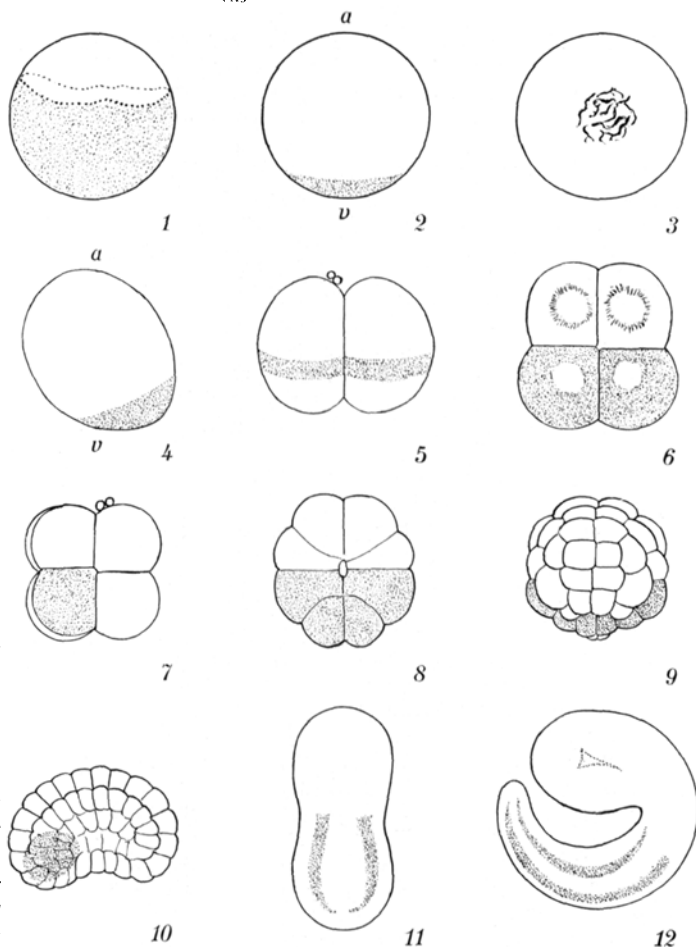
In the Ascidian egg, the distribution of mitochondria in specific cells during the embryonic development has been described by DUESBERG¹. DUESBERG showed that the mitochondria of *Ciona* follow, in their distribution and segregation, the same pattern as the yellow pigment described by CONKLIN² in *Styela*.

Janus green has been used very little to study the differential distribution of mitochondria in different kinds of eggs or in the Ascidian egg itself. Only CHILD³ recently used it in *Clavelina* egg, in the leucobase form after the reduction with sodium hydrosulfite, showing the existence of oxido-reduction gradients or peculiar oxido-reduction patterns.

The present work will be an introduction to more extensive research on the segregation of the mitochondria in the different cells of a developing embryo, and on the rôle of mitochondria in the processes of embryonic differentiation and induction.

(2)–*Phallusia mamillata* eggs were used as material for the present research. They are glass-clear, but an accurate observation reveals in the fertilized egg the yellow crescent, which was described by CONKLIN² in *Styela*. The *Phallusia* egg is therefore very suitable for treatment with vital dyes. The observation is greatly

facilitated in eggs which have been deprived, mechanically or chemically, of their two membranes: the chorial one, with follicular cells, and the inner one, which is very thin and closely adhering to the egg; in the space between the two membranes there are numerous testal cells. Janus green colours the follicular cells purple, the testal cells blue and the egg green. Important differences are observed in the egg before and after fertilization.



Figs. 1–12.—Developing egg of *Phallusia*, after staining with Janus green (see the text). *a* = animal pole; *v* = vegetal pole.

The unfertilized egg possesses at the animal pole—which can thus be very easily determined—a small refringent spot, corresponding to the first metaphasic figure: the nuclear sap of the broken germinal vesicle is spread all over this animal zone. This zone does not take up any colour with Janus green: the rest of the egg, which contains vitelline granules and pigment granules is on the contrary coloured green. The two zones are very sharply separated by a circular line of intensively coloured granules or dots, which are situated subcortically (Fig. 1). The circular line is not even: it is rather sinuous, with slight differences in different eggs. An accurate search for evidence of the existence of a bilateral organization of the egg from the sinuosity of the line did not give positive results. CHILD³ was also unable to find evidence of such an organization with oxido-reduction indicators in the unfertilized *Clavelina* egg.

The aspect of the egg changes completely after the fertilization: the egg gradually becomes clearer, owing to a migration of the green particles towards the vegetal pole: the migration is very perceptible to the eye; it

¹ J. DUESBERG, Bull. Acad. roy. Belgique 5, 463 (1913).

² E. G. CONKLIN, J. Acad. nat. Sci. Philadelphia 13, 1 (1905).

³ C. M. CHILD, Physiol. Zool. 24, 353 (1951).