Aneuploidy as a consequence of senescence and ovariectomy in the golden hamster (*Mesocricetus auratus*)

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Summary. The hypothesis of the preferred X-chromosome loss in elder human females was reevaluated in the golden hamster: early castration of females proved that the increase of aneuploid cells is correlated with the loss of the ovaries. But here, and in old females, aneuploidy consisted of random loss or excess of chromosomes, in no case an X-chromosome.

P. A. Jacobs and co-workers[1] were the first to discover that human somatic cells, especially those of elder individuals, show a tendency towards aneuploidy. This phenomenon increases with age and becomes most evident in women after onset of menopause.

As the overwhelming majority of aberrant cells was found to be monosomic for a chromosome of group X-C, the loss of an X-chromosome was suspected. Arguments for this assumption were the synchrony observed with the decreasing function of the ovaries, as well as the fact that in the individual as a whole monosomy of an autosome is not compatible with life. From this observation, the conclusion was drawn as to the viability of single, nonmalignant cells.

Further research[2-8] has not yet been able to establish conclusively whether the hypoploidy in this specific case is attributable to the loss of an X-chromosome or not.

Materials and methods. For an experimental approach, female Syrian hamsters (*Mesocricetus auratus*) offered 2 appropriate qualities: their reproductive span ends at 1 year of age and they possess morphologically identifiable X-chromosomes. In this species, the X-chromosome is a metacentric and the largest of the whole complement (2n = 44). The experiment was conducted with 3 different groups of female hamsters:

A. The 1st as control group, aged 1 month (earliest ovulation at the age of 28 days),

B. the 2nd group were ovariectomized at the age of 10 weeks in order to establish whether the phenomenon of aneuploidy increasing with age is caused by a decrease of sexual hormones. The chromosome examination of these animals was done 8 months later,

C. the 3rd group, considered senile, included hamsters of 22 and 36 months of age.

Chromosome preparations were made from bone marrow of the femora. The most important innovation of our short term culture consisted of the use of vincristin, as the Syrian hamster has proved to be resistant to colchicine or its derivative colcemide[9-11]. Sachs[12] and Darlington[13], referring to the sympatric distribution of *Mesocricetus auratus* and *Colchicum autumnale*, have put forward the hypothesis that this mammal became tetraploid (most hamster species have 2n around 22) by feeding on the plant with its spindle inhibiting substance!

The yield of well-spread metaphase-figures has been very meagre with colcemide; so vincristin was used. The best results were achieved with 0.5 ml of a 0.004% solution added to 5 ml of the bone marrow suspension. Approximately 50 metaphases of each animal were examined under the microscope, the chromosomes counted and the aberrant cells photographed and analysed again. The percentages of aberrant cells in relation to the total numbers of cells were calculated and compared among the group.

Results and discussion. The frequency of aberrant cells in the control group of young females measured 1.60%, in that of the old animals 16.95%, i.e. 10 times as much. The ovariectomized hamsters had a rate of 13.23% of aneuploid cells. The increased rate of aberrant cells in the ovariectomized and in the old females was mainly caused by hypoploidy (table).

The two categories of hypomodal and hypermodal cells were not caused by the loss of any specific chromosome. There was no case of an X-monosomy or a X-trisomy at all. Errors in this case are hardly possible, since the X-chromosomes are immediately recognizable due to their conspicuous size, as mentioned above.

The further question is which factors or mechanisms lead to this significant loss of chromosomes. If the cause were nondisjunction, one would expect equal numbers of

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Group	Number of animals	Total number of cells counted	Total of aneuploid cells		Hypoploid cells		Cells with 43 chromosomes		Hyperploid cells		Cells with 45 chromosomes	
			n	%	n	%	n	%	n	%	n	%
A: 1 month	5	250	4	1.60	3	1.20	3	1.20	1	0.40	1	0.40
B: ovariectomized, 10 months	15	703	93	13.23	55	7.82	51	7.25	38	5.39	33	4.68
C: 22 or 36 months	17	755	128	16.95	103	13.64	89	11.78	25	3.31	19	2.51

cells with 43 and 45 chromosomes. As far as the ovariectomized animals were concerned, the χ^2-test confirmed this assumption as the resulting $p < 0.2$ is not significant. As the old hamsters showed a predominance of cells with 43 chromosomes over such with 45 the resulting $p < 0.001$ proved to be a highly significant deviation. This points to the fact that nondisjunction alone cannot be responsible for the hypoploidy described above.

One important explanation would be the lack of female sexual hormones. It has been mentioned that a sudden increase of aneuploid cells can also be observed in human females along with the ceasing of sexual hormones. Likewise the hamsters of group II showed a significant increase of aberrant cells compared to the control group, close to the group of senile animals. With these hamsters of group B, a condition comparable to menopause had been prematurely induced by tying off the ovaries (exploration at the time of chromosome-preparation showed complete atrophy). This appears to be strong evidence for the fact that sexual hormones, in some way yet unknown, keep cells from deviating into aneuploidy. Several different factors might be responsible for this restraining effect: it might be caused by a time regulator controlling divisional frequency genetically, and this preventing precipitous cell-divisions which are always accompanied with the risk of nondisjunction, as is known to be the case with tumors. Other possibilities might be hormonal components also acting as a regulative control in a fashion yet unknown. In any case, one should not accuse the X-chromosomes, because they were not lost.

Evidence for rapid speciation in African cichlid fishes

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Summary. Extremely rapid divergence among 7 species of African cichlid fishes is suggested by high estimates of allozymic similarity. Significant differences in gene frequencies among sympatric populations support reproductive isolation of these taxa.

Extreme examples of adaptive radiation and community complexity occur among African fishes of the family Cichlidae. In Lake Malawi, Africa, approximately 300 cichlids are endemic. It has been suggested that this level of taxonomic diversity may have been achieved by fairly rapid rates of speciation[2-4]. In turn, rapid speciation has suggested a variety of novel modes of divergence[5-7]. To examine relative rates of speciation in this fauna, genetic similarity estimates among 7 endemic Malawi cichlids were examined by electrophoresis. Extremely rapid speciation is supported by the low degree of genetic differentiation among these species.

The species examined (table 1) are the most common members of the Mbuna complex a presumably monophyletic group of approximately 30 species generally restricted to the rocky littoral zone. This particular complex is of interest to students of evolution because it occurs in an area which lacks obvious physical barriers usually associated with allopatric speciation[2]. A minimum of 16 allozyme loci encoded by 10 protein systems was examined by standard methods of starch gel electrophoresis from tissue extracts of fish collected sympatrically near Monkey Bay, Malawi[8]. Genetic similarities were computed from estimates of allele frequencies by Nei's Coefficient of Identity (I)[9]. This statistic can vary from 0 to 1, with 1 indicating complete genetic identity. Interspecific similarities in fishes vary widely, but are generally $I < 0.90$ (Sarich[10]). Similarities among the 7 endemic cichlids ranged from 0.85 to 0.99 (table 1; mean 0.934 ± 0.008). Genetic distance between species is apparently a simple function of elapsed time since divergence from a common ancestor[11,12]. Unfortunately, accurate rate constants de-

Table 1. Genetic similarities (I)[9] among 7 endemic Malawi cichlids

		A	B	C	D	E	F	G
Petrotilapia tridentiger	A	–	0.956	0.973	0.983	0.955	0.919	0.906
Labeotropheus fulleborni	B		–	0.903	0.932	0.919	0.864	0.856
Pseudotropheus tropheops	C			–	0.978	0.994	0.923	0.931
P. auratus	D				–	0.966	0.923	0.899
P. zebra	E					–	0.912	0.938
P. elegans	F						–	0.991
P. livingstoni	G							–

Horizontal starch gel electrophoresis was performed on tissue extracts in a standard manner[8]. At least 28 individuals were examined for each species. Genetic similarities were calculated from observed allele frequencies at loci encoded by the following proteins: aminopeptidase, esterase (EST), aldolase, general protein (GP), lactate dehydrogenase (LDH), malate dehydrogenase, phosphoglucose isomerase (PGI), phosphoglucomutase, sorbitol dehydrogenase, superoxide dismutase, and xanthine dehydrogenase.

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