International Journal of Zoology and Applied Biosciences
Volume 3, Issue 4, pp: 362-368, 2018
https://doi.org/10.5281/zenodo.1403240

http://www.ijzab.com

ISSN: 2455-9571

Research Article

IDENTIFICATION OF MEIOTIC (PACHYTENE) CHROMOSOME IN MALE SYRIAN HAMSTER

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Article History: Received 6th August 2018; Accepted 17th August 2018; Published 25th August 2018

ABSTRACT

In many mammals, especially rodents, centric fusions of acrocentric chromosomes constituted the most common mechanism of chromosome structural changes during the course of karyotypic evolution. The present study is an attempt to present here a detailed description of early meiotic stages (i.e. Pachytene, diplotene and diakinetic configurations) with an emphasis on the identification of distinctive features of the elongated chromosome (chromomeric) sequences. This together with a prometaphase idiogram could provide a schematic representation of Syrian hamster meiotic chromosomes, especially of X and Y chromosome behavioral patterns during the early meiosis.

Keywords: Mammals, Chromosomes, Idiogram, Meiosis.

INTRODUCTION

The Golden Syrian Hamsters belonging to the Family Cricetidae fall under the Tribe Cricetini, Subfamily Cricetinae of the Order Rodentia. They are one among most versatile cytogenetic objects in the field of rodent biology. The Syrian Hamsters are more distantly related to the Genus *Ellobius,* rather than to other vibrant members of the Subfamily, especially the Genus, *Microtus.* Order Rodentia is one of the largest orders of mammals constituting about 40% of the recognized mammalian species and is composed of 1700 recent species belonging to 45 families and 360 genera, occupying a wide range of habitats throughout the world (Anderson *et al*., 1967). According to (Achenbach *et al*., 2004), it seems that Palaearctic hamsters (Cricetinae) are the sister group to Arvicolinae at the time of Arvicolinae/ Cricetinae divergence that dates back to 16.3-18.5 million years. Chromosomal variation in the Arvicolinae (Voles) ranges from 2n=17 in *Ellobius lutescens* and *Microtus oregoni* to 2n=62 in some species belongs to Genus *Microtus* (Subgenus *Pitymys* and *Sumarionys*) (Modi & Tayade, 2006; Pankrats, 2006; Volobouev *et al*., 2006). For this group, some cytogenetic mysteries remain unresolved. On the other hand, the subfamily Cricetinae, at present is represented by only 7 genera with 18 species and chromosomal range is quite narrower, when each of the genuses is cytogenetically scrutinized. Most species including some important and principal taxonomic groups have not yet been screened by the modern methods of molecular cytogenetic technology except for a few species.

In recent years, classical cytogenetic studies have transformed dramatically due to an introduction of cross-species chromosome painting studies that have provided essential information for mapping chromosomal homology and thereby enhanced in reconstructing ancestral genomes in mammalian orders (Yang & Ayers, 2003). As is well known that comparative chromosomal painting gives more accurate and fuller information content about interchromosomal rearrangements and breakpoints in karyotypic comparisons of closely related species rather than the conventional chromosome banding approach. However, (Romanenko *et al*., 2007) and (Brodzik *et al*., 2016) have made efforts to provide some insights into the aspects of essential phylogenetic relationships existing between most of the Cricetini species and of genera as well as between hamsters and some representatives of other muroid families. Thus, utilizing appropriate chromosome probes during the cross-species chromosomal paintings to access and to access genome-wise homology search among a few selected voles including the Genus *Microtus* (of Arvicolinae), *Mesocricetus* (of Cricetinae) and *Mus musculus* (of Murinae) and that has allowed some precise

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estimation of chromosome sites indicating homology between four rodents. Furthering upon the same issue, but involving two more tundra voles which differ drastically in their karyotypic organization (2n=17 and 62), and upon using *Mesocricetus* painting probes, that has enabled in delineating chromosome structural variation that could have been excluded thereby lending themselves to construct an ancestral karyotype for the *Ellobius* species group (*E. talpinus* and *E. lutescens*). A considerable number of rearrangements have been detected. It seemed that inversions played a minor role in the genome evolution of these *Ellobius* species and in *Mesocricetus auratus* probes have led to identify 44 and 43 homologous autosomal loci in *E. lutescens* and *E. talpinus* karyotypes respectively.

Differential chromosomal stainings are available for various species belonging to genera in the tribe, Cricetini of the Eurasian Cricetinae including *Cricetus Cricetulus, Tscherskia, Phodopus* and *Mesocricetu* (Roswitha Gamperl *et al.*,1978). Recently, some species of *Allocricetulus,* the Eversmann hamsters of Russian origin, based on differential stainings (G, C and NOR bands) have been described and comparison was made with that of the Genus *Cricetulus*. This implies that these karyotypes have emerged independently from a common ancestral karyotype (2n=34) through chromosome fusion. A similar form of chromosomal reorganization has previously been described for the genera *Cricetus* and *Cricetulus* (Roswitha Gamperl *et al*., 1978). Interestingly, C-stainings of these two taxa revealed that *Cricetus cricetus* shows a large amount of predominantly centromeric heterochromatin where as in *Cricetulus griseus,* C-bands are less conspicuous and in a few chromosomes they do not show any centromeric heterochromatin. In another American creeping vole, *Microtus oregoni,* karyotype and sex-chromosomes reveal differences between two geographical populations (Libbus & Johnson, 1988) and it is interesting to note that whether the observed differences in the relative size of the X chromosome is characteristic of the different geographical populations. Chromosomal changes are undoubtedly due to alterations within the genome resulting in the emergence of new species, although it is not clear whether they are casually related or a consequence of evolution (Fredga & Nawrin, 1977) based upon their experience with rodent cytogenetic research implied to the extent that changes in the Fundamental Number (FN) of a karyotype may be due not only to chromosomal alterations brought about through the involvement of either Robertson an and/or non Robertson an changes, but also due to the effective inclusion or exclusion of constitutive heterochromatin component (especially relevant to certain taxa). *Mesocricetus brandti*, the Turkish hamsters, comprises at least seven populations, referred to as cryptic species each with a different chromosomal constitution. The hamsters from such populations have a diploid number in the range of 42; the chromosomal polymorphisms are due to variable number of acrocentrics. One population of *M. brandti* from the area of Ankara, however, has 44 chromosomes. Prior to the innovations of banding techniques, the karyotyping of *M. brandti* from Kurdistan was reported by Lehman & Macpherson, (1967) to have 2n=42 chromosomes with no acrocentric chromosomes. Popescu and DiPaolo (1980) have reported of animals derived from 200 miles apart within Turkey (Jagiello *et al*., 1992).

Mesocricetus newtoni, the Romanian hamster with 2n=38 using conventionally stained preparations was reported by (Ferlin *et al*., 2007). In their subsequent report has reprimanded of chromosomal derivation processes based on the differentially stained G and C-banded chromosomes. However detailed processes involved in the chromosomal derivation between *M. auratus* and *M. newtoni* was highlighted by Volobouev *et al*., (2006)) having heterochromatinization which might have played important role in such derivation events. In the light of conventional karyotype of different hamster species that became available, speculations arose concerning their evolutionary pathways. One hypothesis suggested that *M. auratus* evolved from two species of Cricetinae that had exactly half the number (Gates & Zimmermann, 1953). Recent chromosome banding analysis of five species of Cricetinae suggested an alternative view upon their evolutionary trend (Gamperl *et al*., 1982). However, (Popescu, *et al*., 1987) have made a comprehensive survey of karyotypes of three species of the Genus *Mesocricetus* based on good G-banding of whole chromosomes, or segments that exists among *M. auratus, brandti* and *newtoni* genome. The euchromatic arm of the X-chromosome and 11 autosomes of each species karyotype suggested similar G-band patterns.

In many mammals, especially rodents, centric fusions of acrocentric chromosomes constituted the most common mechanism of chromosome structural changes during the course of karyotypic evolution (Fredga & Nawrin, 1977). That means either chromosome fusion or fission has played a major role in the pathway of karyotypic evolution increases in case of some rodent karyotypes. However, whether the evolutionary trend for the species of the genus *Mesocricetus* occurred as a result of an increase (38>42>44) or a decrease (44>42>38) in chromosome number is a moot point. Chromosome banding pattern is one criterion for determining the direction of species evolution. The chromosomal derivation process for *M. brandti and M. newtoni* karyotype is not prevailed upon *M. auratus* complement was identified and further the findings deemed to support the interpretation that *M. brandti, and M. newtoni* developed independently from *M. auratus* (Popescu *et al*., 1987) karyotype. The Y chromosomes are also unequal. *M. auratus* has the largest Y; *newtoni* has a Y with slightly shorter long arm, while *brandti* has the smallest Y with both long and short arms containing less heterochromatin. Therefore, the breakage at different points of *auratus* X heterochromatic arms and the Y with deletions of heterochromatic segments could account for the reduced size of the *brandti* and *newtoni* sex chromosomes. The present study is an attempt to present here a detailed description of early meotic stages (i.e. pachytene diplotene and diakinetic configurations) with an emphasis on the identification of distinctive features of the

elongated chromosome (chromomeric) sequences. This together with a prometaphase ideogram could provide a schematic representation of Syrian hamster meiotic chromosomes, especially of X and Y chromosome behavioral patterns during the early meiosis.

MATERIAL AND METHODS

Sample collection

The Syrian Golden hamsters were procured from a local animal supplier, in Bangalore. Nothing of their genetical background is known but judging of their appearance and behavior, they were typical representatives of this species. The animal has been dissected for bone marrow and liver for mitotic preparations and testis for meiotic preparations. For the cytological preparations the protocols given by Hungerford & Hungerford, (1978) were followed.

Processing of cell suspension

The required material was dissected on to the hypotonic solution (KCI 0.1M; 0.125M; and 0.150M for meiotic cells and 0.075M for somatic cells) and was thoroughly minced with the help of a small scissor with curved tip until a fine cell suspension was obtained. After the mincing was completed, the cell suspension was transferred by a Pasteur pipette into a centrifuge tube and the cells were sediment by centrifugation at 750 rpm for 5 minutes. The hypotonic solution (supernatant) was then removed and replaced with 3-5ml of freshly prepared mixture of absolute methanol and glacial acetic acid (3:1) (fixative). The pellet of cells was then dispersed in the fixative by gentle agitation with the help of a Pasteur pipette and the volume was slowly increased (3-5 ml) by the addition of more fixative. The cell suspension was further centrifuged and resuspended with one more change in fresh fixative. After the last change, a small volume of fixative was again added to obtain a turbid cell pellet.

Preparation of slides

A Coplin jar containing slides immersed in absolute alcohol was pre-refrigerated. Two more Coplin jars (No.1 and 2), each contain distilled water were also pre-refrigerated for some time prior to slide making. The slide was then transferred to Coplin jar number 2 which was also shaken well. A Pasteur pipette was used to drop 3 or 4 drops of processed cell pellet over the wet slide held in an inclined angle. The slide was then shaken vigorously to remove excess liquid accumulated over the surface and air-dried on a slide warmer at 40 c for 1-2 minutes.

Conventional Giemsa staining

This procedure was adopted to observe the mitotic index in somatic tissues and meiotic stage from the gonadal tissues after slide preparation by air drying method. The prepared slides were immersed in the diluted Giemsa staining solution. The staining solution was prepared by adding 1 ml of Sorenson's phosphate buffer (pH at 6.8) and 1 ml Giemsa stock solution to 48ml distilled water. The slides were stained for about 5 minutes and rinsed briefly in distilled water before air-drying over a slide warmer at 60℃.

G-banding technique-ASG procedure

The method of (Sumner & Robinson, 1976) was adopted. Air-dried slides were incubated for one hour in 2xSSC (0.3M sodium chloride and 0.03 tri-sodium citrate of pH 6.8-7.0) at 65 C in a water bath. After incubation, the slides were rinsed briefly in distilled water at room temperature and stained for about 5 minutes in a diluted Giemsa stock solution and Mcllvaine's buffer at pH 6.8, which was diluted in 96ml distilled water. Finally, the slides were again briefly rinsed twice in deionized water and air-dried. Further chromosomal analysis was made on unmounted slides.

G-banding technique-trypsin treatment

A slightly modified method of (Seabright, 1971) was followed. Trypsin solution (0.25%) was diluted with Hank's solution without Ca++ and Mg++. Slides were kept horizontally with the cell suspension facing upwards and flooded with diluted trypsin solution for 30-90 sec before the solution was drained-off. The slides were then dipped twice in the physiological saline solution and air-dried. The air-dried slides were observed under a phase-contrast microscope prior to staining (1ml Giemsa stock solution and Sorenson's buffer with 97ml distilled water) for less than a minute and air-dried.

C-banding technique

For the staining of constitutive heterochromatin, the procedure of (Sumner & Robinson, 1976) was adopted. The slides were treated for 1 hour with 0.2N HCl at room temperature and then briefly rinsed in distilled water. The slides were then treated for 4-5 minutes with 5% aqueous solution of barium hydroxide octahydrate at 50 C which was followed by a thorough washing in slow running tap water for few minutes. The slides were then incubated for 1-4 hours in 2x SSC solution at 60 c and stained for 1-2 hours in diluted 2% Giemsa solution (Phosphate buffer pH at 6.8)

Silver-staining technique

Silver-staining was carried out according to the modified technique of (Kohno & Roth, 1978). The slides were first pretreated in a buffer solution (0.1M, pH 9.0) for 5-20 minutes at room temperature and then stained for 24-50 hours at 50 c in a Coplin jar containing 50% $AgNO_3$ solution. The slides were subsequently counter stained with a diluted (2%) Giemsa solution.

De staining

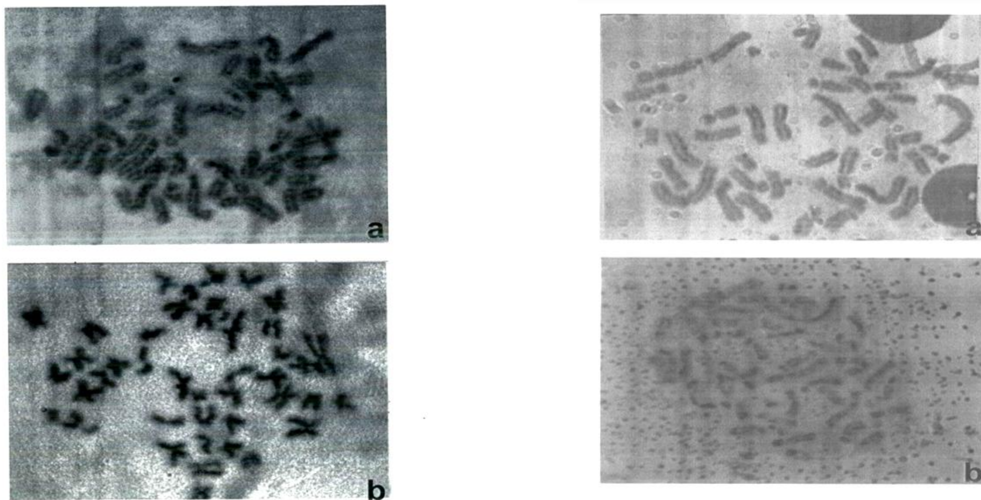
For destaining, the slides were dipped in xylene, then air-dried and was placed in a mixture of xylene and absolute alcohol (1:1) for 5 minutes in each case. The slide was then passed through different grades of alcohol consisting of 95, 90 and 80% for 5 minutes in each case before it was finally passed through 70% alcohol for 60 min and air-dried.

Chromosome photography

All light photomicrography was done with a Zeiss photomicroscope III equipped with 35mm camera, on 35mm ORWO 125 ASA film. All fluorescence photomicroscopy was done by the same microscope, with a zeiss epifluorescence illuminator and a 35mm camera using ORWO 80 ASA negative film.The negatives were processed in a small day light developing tank following the conventional photographic procedures. The positive prints were made on AGFA-kodabromide printing paper of different grades depending on the contrast needed.

RESULTS AND DISCUSSION

Several G-banded *M. auratus* metaphase karyotype (2n=44) are available (Figure 1). In addition to G-banded (ASG-as well as trypsin treated) metaphase Chromosomes, some preparations were also stained by Aceto-Orcein solution for the purpose of preparation of individual karyotype. Prometaphase chromosomal Compliments were selected for the purpose of preparing comparable and complete Karyotype so as to extrapolate richer details of G-banding delineation. Both Giemsa and Orcein stained preparation reveal detailed but similar structural organization thereby reinforcing accurate identification of individual chromosomes in the karyotype (Figure 1).

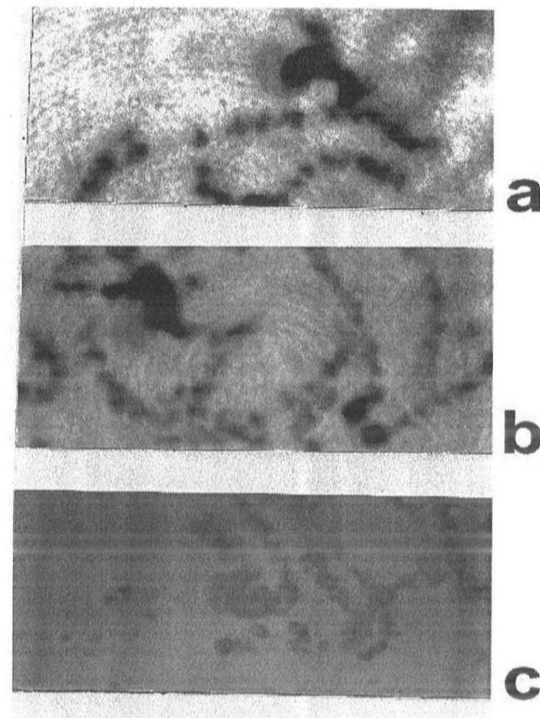


a. ASG banded male mitotic metaphase compliment.

b. Orcein stained female mitotic metaphase complimen.

a. Conventional Giemsa stained female metaphase karyotype.

b.ASG=banded female metaphase karyotype.



a.NOR stained pachytene chromosomal compliment.

b.C-banded pachytene chromosomal compliment. C. nor –stained interphase nuclei.

Figure 1. Identification of individual chromosome in the karyotype methods.

Currently available cytological techniques make it possible to observe all stages of Meiosis, especially pachytene, diplotene and I and II metaphase the importance of meiosis ,ie, in view of defining the modalities of crossing over, pairability and in the segregation patterns of homologous chromosomes in the earlier meiotic processes offer excellent opportunities to study the progression of chromosome mechanics for this purpose, male meiosis provides a realistic and a convenient means of study. In recent years, decisive progress has been made in obtaining both Well- spread pachytene bivalents and in the presentation of their chromomeric structure following the introduction of testicular material to a prolonged hypotonic treatment of low molecular weight salt solutions (especially KCl), prior to chromosome preparations (Cadoz *et al*., 1984; Jhanwar *et al*., 1982).This technique known as the "Chromomere mapping" technique permits suitable pachytene karyotype to be obtained. This has enhanced in elaborating each autosomal bivalent identified according to the number and gradualsequencing of their chromosomes in a bivalent.

A characteristic XY body (sex vesicle) is not apparent prior to pachytene, however, in earlier stages, heterochromatic bodies corresponding in size to the heterochromatic segments of sex chromosomes are seen. These chromocenters may lie near each other or far apart. This body shows some structural differentiation during early and mid pachytene but by late pachytene it has become more oval and dense and structural details are no longer evident. A small heteropynotic block corresponding to an autosomal bivalent is frequently observed adjacent to the XY body during pachytene thus unraveling of the chromosomal threads in the XY body occurs during diplotene, but no definite arrangement of the chromosomes could be established at this stage. Quite a good number of prints of metaphase I were analyzed with regard to the relationship between the X and Y chromosomes. The Y chromosome is easily identified as a large chromosome associated with the X (Figure 1a).

Although the sex chromosomes were always near or touching each other chiasmata were never observed. Unequivocal end-to-end associations were observed in 23 metaphases and lateral associations were observed in 27 cells. Analysis of arms involved in these associations was not performed in this sample of 57 metaphases stained with Giemsa solution Figure 1). In C- banded preparations, the XY body in pachytene cells is strongly heterochromatic (Figure 1 and 2) but individual chromosome arms can not be identified. In C-banded metaphase the X and Y chromosomes exhibit the same C-banding as in mitotic cells.

It is believed that the centromere of the X is situated between the condensed regions and elongated regions. It is difficult to judge the real length of the despiralized part of the X, as has been discussed among others by Mathey & Bensoam, (1971), However, postulating that the

isopycnotic parts of X and Y have the same degree of contraction, the Y appears to be slightly longer than the isopycnotic_part of-of the X. Consequently, this arm of the X is the shorter one since the long arm of the X is longer than the whole of Y. A good correspondence was observed between the major chromomeric dark bands along the pachytene bivalent and the metaphase G-banded dark bands standardized from the earlier studies based on mitotic metaphase chromosomes of median contraction (Figure 1b). Although the number and sequences of chromomeres and the sequencing of G-bands are approximately the same,some differences were noticed concerning to position of chromomeres along some bivalents, and of stretching of some interchromeric regions.Each bivalent is much longer and has a larger nucleus of minor chromomere than its counter part corresponding to mitotic metaphase G-bands. This situation was found most ideal since meotic chromosomes offer more realistic picture of genomic organization in its natural context. Our selection of elongated pachytene chromosomes for the said specific purposes, unambiguous identification of individual chromomeres in the complemen through their chromomeric sequences has thus enabled in the preparation of complete autosomal pachytene chromomeres (Figure 1c).

In the light of prevalence of a striking correspondence in the number and sequential ordering of chromosome sequences was evident between pachytene chromomeres and somatic G-bands. Hence no attempt was made to present a detailed presentation and explanation of chromomeric sequences along the individual bivalent. Instead, a diagrammatic representation between the two chromosome types is presented. Positive arm identification could be made is too low to permit conclusions as to the regularity of end attachments between specific arms at metaphase I (Figures 1).

As the majority of the spermatocytes analyzed by light microscopy were from colchicines injected animals, no anaphase I was observed. Proliferation of new differential staining techniques upon delineating chromosome sub structure in recent past has, facilitated in precise identification of individual members in a complement and thus bringing chromosome banding technique a much awaited event in the facets of evolutionary cytogeneticists, the application of these techniques has contributed significantly to our understanding of the taxonomic relationships of a large number of closely as well as distantly related species and has also helped in the precise identification of several doubtful species and in elucidating several substructures of chromatin.

Some of the recent studies on the cytogenetics of mammals, for example, in particular of rodents pertaining to the role of structural rearrangements with the karyotype (Baker & Bickham, 1986; Patton & Sherwood, 1983). Conventional chromosome banding techniques have established many chromosome homologies in closely related species but have failed to define the smaller conserved elements within the human and in other mammalian karyotypes that have been predicted by linkage analysis. As the linkage maps of various species have become more clearly defined, analysis of the linkage disruptions caused by chromosome rearrangements has favored the hypothesis that many chromosomal segments containing conserved linkages may have been preserved through the evolution of mammals (Nadeau & Taylor, 1984; Sawyer, 1994). Therefore, high-resolution chromosome banding patterns in the mammalian should, in theory, contain conserved segments that have remained intact during evolution. In fact, comparison of late prophase chromosomes in regions of supposed genetic homology has shown that some banding relationships have remained intact in man and mouse during mammalian evolution (Sawyer) and (Hozier, 1986),as well as in intermediate species ,such as the cat, monkey, oranguton, gorilla and chimpanzee (Nash & O Brien, 1982; Yokoyama *et al*., 2017).

CONCLUSION

Identification of meiotic (pachytene) chromosome in male syrian hamster the use of high resolution comparative cytogenetics in conjugation with data on the regional localization of genes by *in situ* hybridization within apparently similar banding patterns of these various species supports the concept of conserved chromosome regions. This together with a prometaphase idiogram could provide a schematic representation of Syrian hamster meiotic chromosomes, especially of X and Y chromosome behavioral patterns during the early meiosis.

ACKNOWLEDGEMENT

The authors are thankful to the Co Centre for Applied Genetics, Department of Zoology, Bengaluru University, Bengaluru for providing laboratory facilities.

REFERENCES

Achenbach, S., Moselewski, F., Ropers, D., Ferencik, M., Hoffmann, U., MacNeill, B., Jang, I.K. (2004). Detection of calcified and noncalcified coronary atherosclerotic plaque by contrast-enhanced, submillimeter multidetector spiral computed tomography: a segment-based comparison with intravascular ultrasound. *Circulation, 109*(1), 14-17.

Anderson, I., Burnett, G., Geddes, W., Jones, A., Parsons, D., Roberts, D., Meybeck, A. (1967). 10. Studies on poly (ethyl acrylate) in theta solvents. *European Polymer Journal, 3*(2), 161-320.

Baker, R.J., & Bickham, J.W. (1986). Speciation by monobrachial centric fusions. *Proceedings of the National Academy of Sciences, 83*(21), 8245-8248.

Brodzik, K., Faber, J., Gołda Kopek, A., & Lomankiewicz, D. (2016). Impact of multisource VOC emission on in-vehicle air quality: test chamber simulation. Paper

presented at the IOP Conference Series: Materials Science and Engineering.

Cadoz, C., Luciani, A., Florens, J.-L., Roads, C., & Chadabe, F. (1984). Responsive input devices and sound synthesis by stimulation of instrumental mechanisms: The cordis system. *Computer Music Journal, 8*(3), 60-73.

Ferlin, A., Raicu, F., Gatta, V., Zuccarello, D., Palka, G., & Foresta, C. (2007). Male infertility: role of genetic background. *Reproductive Biomedicine Online, 14*(6), 734-745.

Fredga, K., & Nawrin, J. (1977). Karyotype variability in *Sorex araneus* L.(Insectivora, Mammalia). *Chromosomes today, 6*, 153-161.

Gamperl, R., Ehmann, C., & Bachmann, K. (1982). Genome size and heterochromatin variation in rodents. *Genetica, 58*(3), 199-212.

Gamperl, R., Vistorin, G., & Rosenkranz, W. (1978). Comparison of chromosome banding patterns in five members of Cricetinae with comments on possible relationships. *Caryologia, 31*(3), 343-353.

Gates, R.R., & Zimmermann, A.A. (1953). Comparison of skin color with melanin content1. *Journal of Investigative Dermatology, 21*(6), 339-348.

Hozier, J. (1986). High resolution of mouse chromosomes: banding conservation between man and mouse. *Science, 232*(4758), 1632-1635.

Hungerford, D., & Hungerford, A. (1978). Chromosome structure and function in man. *Cytogenetic and Genome Research, 21*(4), 212-230.

Jagiello, G.M., Fang, J.S., Hembree, W.C., & Ducayen Knowles, M.B. (1992). Some characteristics of oogenesis in the female Turkish hamster subjected to modified environments. *Environmental and Molecular Mutagenesis, 19*(4), 316-322.

Jhanwar, S., Burns, J., Alonso, M., Hew, W., & Chaganti, R. (1982). Mid-pachytene chromomere maps of human autosomes. *Cytogenetic and Genome Research, 33*(3), 240-248.

Kohno, T., & Roth, J. R. (1978). A Salmonella frameshift suppressor that acts at runs of A residues in the messenger RNA. *Journal of Molecular Biology, 126*(1), 37-52.

Lehman, J. M., & Macpherson, I. (1967). The karyotype of Kurdistan hamster. *Journal of Heredity, 58*(1), 29-31.

Libbus, B., & Johnson, L. (1988). The creeping vole, *Microtus oregoni*: karyotype and sex-chromosome differences between two geographical populations. *Cytogenetic and Genome Research, 47*(4), 181-184.

Mathey, F., & Bensoam, J. (1971). Nouvelle methode de fluoration des groupements carbonyles utilisant l'hexafluorure de molybdene. *Tetrahedron, 27*(16), 3965-3969.

Modi, A., & Tayade, P. (2006). Enhancement of dissolution profile by solid dispersion (kneading) technique. *AAPS Pharmscitech, 7*(3), E87.

Nadeau, J. H., & Taylor, B. A. (1984). Lengths of chromosomal segments conserved since divergence of man and mouse. *Proceedings of the National Academy of Sciences, 81*(3), 814-818.

Nash, W. G., & O'Brien, S. J. (1982). Conserved regions of homologous G-banded chromosomes between orders in mammalian evolution: carnivores and primates. *Proceedings of the National Academy of Sciences, 79*(21), 6631-6635.

Pankrats, A. (2006). A. Pankrats, G. Petrakovskii, V. Tugarinov, K. Sablina, L. Bezmaternykh, R. Szymczak, M. Baran, B. Kundys, and A. Nabialek, J. Magn. Magn. Mater. 300, e388 (2006). *Journal of Magnatic Materials., 300*, e388.

Patton, J. L., & Sherwood, S. W. (1983). Chromosome evolution and speciation in rodents. *Annual Review of Ecology and Systematics, 14*(1), 139-158.

Popescu, N., DiPaolo, J., & Amsbaugh, S. (1987). Integration sites of human papillomavirus 18 DNA sequences on HeLa cell chromosomes. *Cytogenetic and Genome Research, 44*(1), 58-62.

Romanenko, S.A., Volobouev, V. T., Perelman, P.L., Lebedev, V.S., Serdukova, N. A., Trifonov, V.A., Bulatova, N.S. (2007). Karyotype evolution and phylogenetic relationships of hamsters (Cricetidae, Muroidea, Rodentia) inferred from chromosomal painting and banding comparison. *Chromosome Research, 15*(3), 283-298.

Sawyer, E. (1994). Melt segregation in the continental crust. *Geology, 22*(11), 1019-1022.

Seabright, M. (1971). A rapid banding technique for human chromosomes. *Lancet, 2*, 971-972.

Sumner, A., & Robinson, J. (1976). A difference in dry mass between the heads of X-and Y-bearing human spermatozoa. *Journal of Reproduction and Fertility, 48*(1), 9-15.

Volobouev, V., Gallardo, M., & Graphodatsky, A. (2006). Rodents cytogenetics. *Atlas of Mammalian Karyotypes*, 173-176.

Yang, W., & Ayers, P.W. (2003). Density-functional theory *Computational Medicinal Chemistry for Drug Discovery* , 103-132.

Yokoyama, C., Sueyoshi, Y., Ema, M., Mori, Y., Takaishi, K., & Hisatomi, H. (2017). Induction of oxidative stress by anticancer drugs in the presence and absence of cells. *Oncology Letters, 14*(5), 6066-6070.