telomeric heterochromatin has been recorded in chromosome pairs Nos 1, 3, 4 and 10. An extended and very intense heterochromatic block has been noted in the pericentromeric region of one of the homologues of chromosome pair 7 (figs 1,a and 2) denoting the presence of a secondary constriction in this particular region. By analyzing the C-band karyotype of the female specimens and by comparing the same with that of the male we designated one of the smallest biarmed chromosome pairs as the ZW sex chromosome pair. This pair exhibitied a clear heteromorphism in C-band preparations. The W chromosome is distinguishable by its dense and almost completely C-band positive staining (figs 1,a and b and 2). The Z chromosome, on the other hand, has only inconspicuous centromeric heterochromatin.

Attempts to demonstrate heterochromosomes in Anura were not convincing[16], until Schmid[10] showed by C-band staining the existence of a highly differentiated ZW sex chromosome pair in female *Pexicephalus adspersus* (= *Rana adspersa*). The W chromosome in this species is considerably smaller than the Z, and approximately half of it is composed of constitutive heterochromatin. In *Rana tigrina* we find no size difference, but the W chromosome is composed almost wholly of C-band positive constitutive heterochromatin. So far as we are aware, this is the 1st report on a completely heterochromatic sex (W) chromosome in Amphibia which thus resembles the W chromosome of most snakes and birds[17,18].

Two contradictory schools of opinion try to explain the mechanism of sex chromosome differentiation in vertebrates. According to Ohno[19] and others the morphological differentiation of Z and W (or X and Y) chromosomes evolves through an initial chromosomal rearrangement. Contrary to this, Singh et al.[20] gave priority to an initial heterochromatinization phenomenon. The occurrence of 2 unequal-sized sex chromosomes with an only partially heterochromatinized W in *Rana adspersa*[10] provided evidence in favor of Ohno's hypothesis, whereas the present finding in *Rana tigrina*, of an almost completely heterochromatic W of equal size, may strengthen the view of Singh et al.[20]. Further investigations on Anura should contribute to elucidating the question.

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A tertiary trisomic interchange heterozygote in pea (*Pisum sativum* L.)

V.C. Mercykutty and H. Kumar[1,2]

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005 (India), October 7, 1981

Summary. In M_2 generation of an interchange heterozygote, one off-type plant was isolated which was characterized by the presence of weak and slender stems, with profuse semi-spreading branching, narrow and yellow-green foliage, small and weak pods, and 10 days late-flowering and -maturing as compared to wildtype. The mutant had high pollen and ovule sterility (76-80%) and showed $5^{II}+2^{II}$ (heteromorphic) + 1^{I} (small chromosome) in 85% of the cells. The mutant appeared to be a tertiary trisomic interchange heterozygote. The possible mechanism of the formation of heteromorphic bivalents and a small chromosome are discussed.

Although a few trisomics in pea, primary and tertiary, have been isolated by several workers[3-12], a tertiary trisomic interchange heterozygote (i.e., tertiary trisomic in interchange heterozygote background) seems not to be known yet. The present note deals with the cytomorphological behavior of one such mutant.

One off-type plant was isolated in M_2 generation of the selfed progeny of an interchange heterozygote which was induced through gamma-ray irradiation (10 krad) of the F_1 seeds from the parents, 68 C (from Dr. W. Gottschalk, FRG) and 5064-S (a selection from the original mutant line 5064 - from Dr S. Blixt, Sweden). This parental interchange heterozygote was morphologically similar to normal but had pollen and seed sterility about 83 and 88%, respectively. At metaphase I, the interchange heterozygote had bivalents ($5^{II}+2^{II}$, heteromorphic), quadrivalents ($5^{II}+1$

chain of 4) and univalents (2-4) in 78, 12 and 10% cells, respectively, suggesting the presence of an interchange involving 2 nonhomologous chromosomes. Presence of heteromorphic bivalents clearly indicated the exchange of unequal segments. At anaphase I, 3:1 non-disjunction (13%) and laggards (10%) were noticed, as were a few micro-nuclei at telophase II.

The mutant plant was dwarf with yellow-green foliage and was characterized by weak and slender stems, profuse semi-spreading branches, narrow leaflets and stipules, small and weak pods, and 10 days late-flowering and -maturing, as compared to its normal (figs 1 and 2). The mutant showed high pollen (76.5%) and seed (80.5%) sterility and as such produced only a few selfed seeds. At metaphase I, the mutant showed $5^{II}+2^{II}$ (heteromorphic) $+1^{I}$ (small chromosome) in about 85% cells, while 3 to 5 univalents were observed in other cells. None of the cells showed higher association. It was interesting to note 2 heteromorphic bivalents (fig. 4, double bar arrowed) like those present in parental interchange heterozygotes as compared to normal bivalents (fig. 3) and normal division in the small extra chromosome (figs 8 and 9). At anaphase I about 56% cells showed abnormalities; laggards involving both normal chromosomes as well as the small extra chromosome were equally frequent (figs 6 and 7) resulting in abnormalities at subsequent stages (fig. 9).

This mutant thus appeared to be a tertiary trisomic interchange heterozygote-i.e., tertiary trisomic in interchange heterozygote background as a result of 3:1 non-disjunction in the interchange heterozygote in M_1 generation (fig. 10). The extra chromosome in the mutant appeared to be an

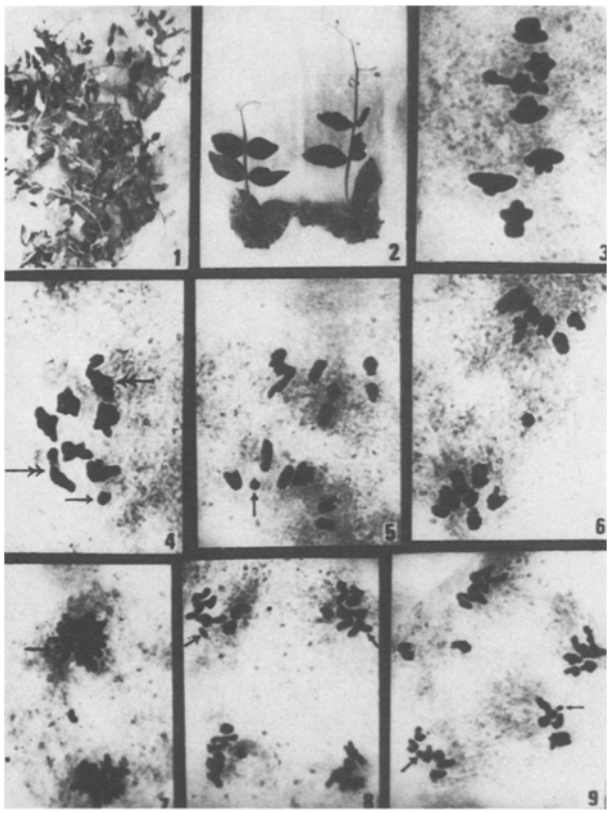

Figure 1. Tertiary trisomic interchange heterozygote, weak and slender stem, profuse semi-spreading branches.

Figure 2. Leaf, normal diploid (left) and tertiary trisomic interchange heterozygote (right) with narrow leaflets and stipule.

Figure 3. Metaphase I, diploid with 7^{II} (about × 1125).

Figures 4–9. Meiotic stages in tertiary trisomic interchange heterozygote (about × 850).

Figure 4. Metaphase I, $5^{II}+2^{II}$ (heteromorphic, double bar arrowed) $+1^{I}$ (small extra chromosome, arrowed).

Figure 5. Anaphase I, normal movement of small extra chromosome (arrowed).

Figure 6. Anaphase I, lagging small extra chromosome.

Figure 7. Normal movement of small extra chromosome with one laggard (normal chromosome).

Figure 8. Anaphase II, normal division of small extra chromosome.

Figure 9. Anaphase II, normal division of small extra chromosome with lagging normal chromosomes.

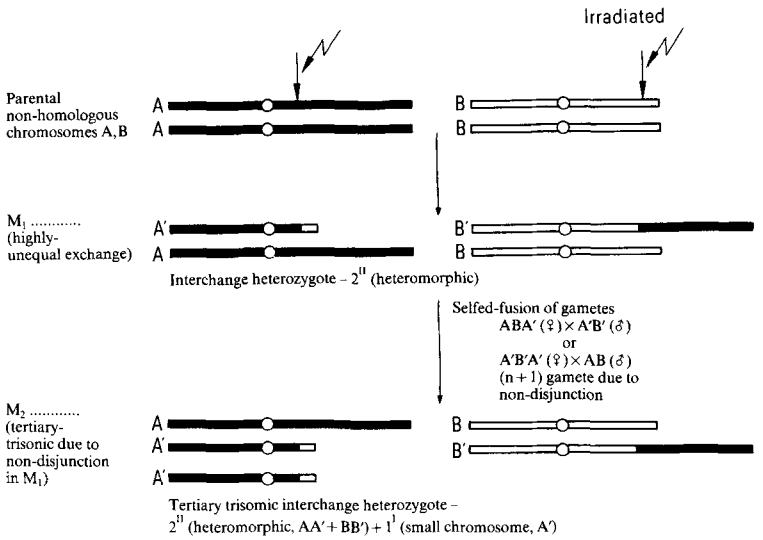

Figure 10. Isolation of a tertiary trisomic interchange heterozygote.

interchanged one. Its small size was probably the result of a highly unequal exchange of segments (i.e., a large segment of a chromosome was exchanged with a very small segment of an other nonhomologous chromosome). Unequal exchanges in heterozygous conditions led to the formation of 2 heteromorphic bivalents, as noted in the interchange heterozygote in M_1, as well as in the mutant in M_2. In these heteromorphic bivalents, tail-like structures in both closed and open bivalents represent the unpaired nonhomologous segments (fig. 4). Observations on low frequency of quadrivalents (12%) in the interchange heterozygote (M_1) and absence of higher association in the mutant are parallel and in conformity with the other reports[13,14] concerning similar situations, that is interchanges involving highly unequal exchange of segments between nonhomologous chromosomes. In addition, the extra chromosome (interchange type) in the mutant might be also partly responsible for the absence of higher association.

Thus, the chromosome constitution of the mutant (tertiary trisomic interchange heterozygote) could be presented as 5^{II} (normal) + 2^{II} (heteromorphic) + 1^{I} (interchanged chromosome with reduced size). Studies with regard to the chromosomes involved, the transmission rate of the extra chromosome and the breeding behavior of the mutant (selfed and F_1 seeds produced by crossing with normal including reciprocal) are being made.

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Teratogenic effects of azaserine in the Syrian golden hamster[1]

B.D. Roebuck and S.J. Carpenter

Department of Pharmacology and Toxicology, and Department of Anatomy, Dartmouth Medical School, Hanover (New Hampshire 03756, USA), July 13, 1982

Summary. Azaserine is a potent pancreatic carcinogen in the rat, but not in the Syrian golden hamster. The present study shows, however, that the hamster shares with the rat susceptibility to the embryotoxic and teratogenic effects of this drug.

Azaserine (O-diazoacetyl-L-serine), a structural analog of glutamine, was originally synthesized and used as an antitumor agent in the early 1950's[2-5]. Although the teratogenic properties of this drug on chick and rat embryos were soon demonstrated[6-8], only recently has it been shown to also have potent carcinogenic effects on the exocrine pancreas of the rat and other laboratory rodents[9]. Interestingly, the Syrian golden hamster was found to be resistant to the carcinogenic effects of azaserine[10]. No explanation for this resistance to azaserine carcinogenicity has yet been found, but in vivo studies indicate that the damaging effect of this drug on DNA of pancreas is similar in the rat and hamster[11,12]. The differential refractoriness of the hamster to the carcinogenic effects of azaserine encouraged us to investigate if this rodent was also resistant to the teratogenic properties of this drug.

Methods. Timed-pregnant Syrian golden hamsters of a standard, commercially available outbred line (Lak:LVG, Charles River Breeding Laboratories, Wilmington, Massachusetts, USA) were used. These were caged individually, given free access to food and water, and housed in a controlled environment room (14 light/10 dark h cycle) until used. In the morning (08.00 h) of the 8th gestational day (the beginning of the teratogenic 'critical period' in this species) hamsters of the experimental group were anesthetized with pentobarbital sodium (50 mg/kg) and given a single i.v. (lingual vein) or i.p. injection of a sterile solution of azaserine (Calbiochem-Behring, LaJolla, California, USA) at dose levels ranging from 2.0 to 3.0 mg/kg b.wt. Matching control hamsters were anesthetized and given i.v. or i.p. injections of sterile saline in volumes equivalent to those administered to the experimental animals ($\simeq$ 0.5 ml). All experimental and control hamsters were sacrificed under deep anesthesia on the 12th gestational day (beginning of the fetal period). Gravid uteri were removed from the animals and the number of gestation sacs (implantation sites) in each recorded. The uteri were then incised and counts made of the number of viable and dead fetuses recovered. Fetuses delivered live were examined, both in the fresh state and after preservation in Bouin's fixative, for gross external abnormalities only.

Results and discussion. Azaserine-treated pregnant hamsters displayed no outwardly obvious signs of toxicity. Their reproductive performance compared to controls is summarized in table 1. In azaserine-treated dams, the fetal death rate (resorptions and stillbirths) increased steeply over the narrow dose range of 2.0 to 3.0 mg/kg. However, differences in fetal death rates between dams injected by the i.p. vs the i.v. route were not statistically significant. The incidence of abnormalities in live fetuses delivered from the different groups of azaserine-treated dams ranged from 9 to 100%. No consistent dose or administration route