Identification of chromosome segments and structural heteromorphism in *Cocculus villosus* DC.

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Application of modified orcein banding technique aided identification of chromosome segments in female *Cocculus villosus*. Heteromorphic pairs in relation to banding pattern could be distinguished in all female plants studied. Cryptic structural heterozygosity is indicated. Correlation with sex is under study.

THE identification of chromosome segments has been much facilitated in recent years through the study of Giemsa-stained bands in chromosomes. Compared to the mammalian system, the response of plant chromosomes to the banding technique is rather meagre[1-10]. In recent years orcein-bands too have been utilized for locating differentiated chromosome segments[11, 12]. Such differentiations in the plant system mostly involve C-bands restricted either to the centromeric or telomeric loci, or occasionally, intercalary segments. In addition to Giemsa C-bands, feulgen-stained bands have also been reported in a few cases in plants[3, 5].

However, one of the serious limitations of the banding technique in plants is the difficulty of securing the manifestation of identical chromosome segments in all homologous pairs. Moreover, most of these techniques worked out so far do not resolve clearly the intercalary bands so essential for identifying the fine structures of chromosomes. The present paper deals with the application of a new method in bringing out structural heteromorphism in species of Menispermaceae.

In order to work out the intercalary banding patterns in chromosomes, root-tips of *Cocculus villosus* DC., a species of the family Menispermaceae were pretreated for 3 h in saturated aqueous mixture of paradichlorobenzene and aesculine at 14–16°C and fixed overnight in acetic–ethanol (1:3). The fixed root tips were hydrolysed in 1 N HCl for 10–12 min at 60°C. After thorough washing, the root tips were kept in 45% acetic acid for 2–3 min and then stained in 2% acetic-orcein for 2–3 min at 60°C. The root tips were then kept in the stain for 3 h at room temperature and finally squashed in a drop of 45% acetic acid. This technique, worked out recently[13], does not require the use of Trypsin or SSC as utilized in earlier papers[11, 12] to secure orcein banding.

Somatic chromosome number is $2n=26$ in this species. There are two pairs of chromosomes (pair nos.1 and 6) with secondary constrictions. Most of the chromosomes are nearly metacentric to submetacentric in nature. All chromosomes show dark bands at the telometric end of both arms except chromosome pair nos. 9 and 10. Pair no. 1 shows two dark bands and one light band on the long arms. The chromosome pair nos. 2, 3, 9 and 10 are heteromorphic for orcein-bands (Figure 1, see next page). The bands differ in relation to size, positions and stainability. The pairs 4, 5, 7, 11 and 12 are homomorphic. But even then, the thickness and gap between the telomeric and intercalary bands on the long arms differ considerably among these pairs (Figure 1).

The idiogram on which the heterochromatic bands are represented allows an easier identification of the chromosomes.

The technique adapted in the present study utilizes only acid treatment for the manifestation of orcein-positive bands in the chromosomes. The orcein bands involve interaction with the nucleoprotein linkage in the chromosomes[11, 12]. As with Trypsin and SSC, prolonged acid treatment at 60°C too possibly affects this protein nucleic acid bonding.

The consistent presence of such heteromorphic pairs in all female plants of a population so far examined indicates clearly a balanced but cryptic heterozygosity in nature. The maintenance of such heterozygosity may suggest some selective value. Population studies from different areas may further indicate the extent to which populations differ in relation to such heterozygosity. The efficacy of the technique in bringing out intercalary bands and structural heteromorphism makes it an important tool in the analysis of cryptic chromosomal polymorphism. Efforts are under way to identify the chromosome segments in male plant and its relationship with the female counterpart.

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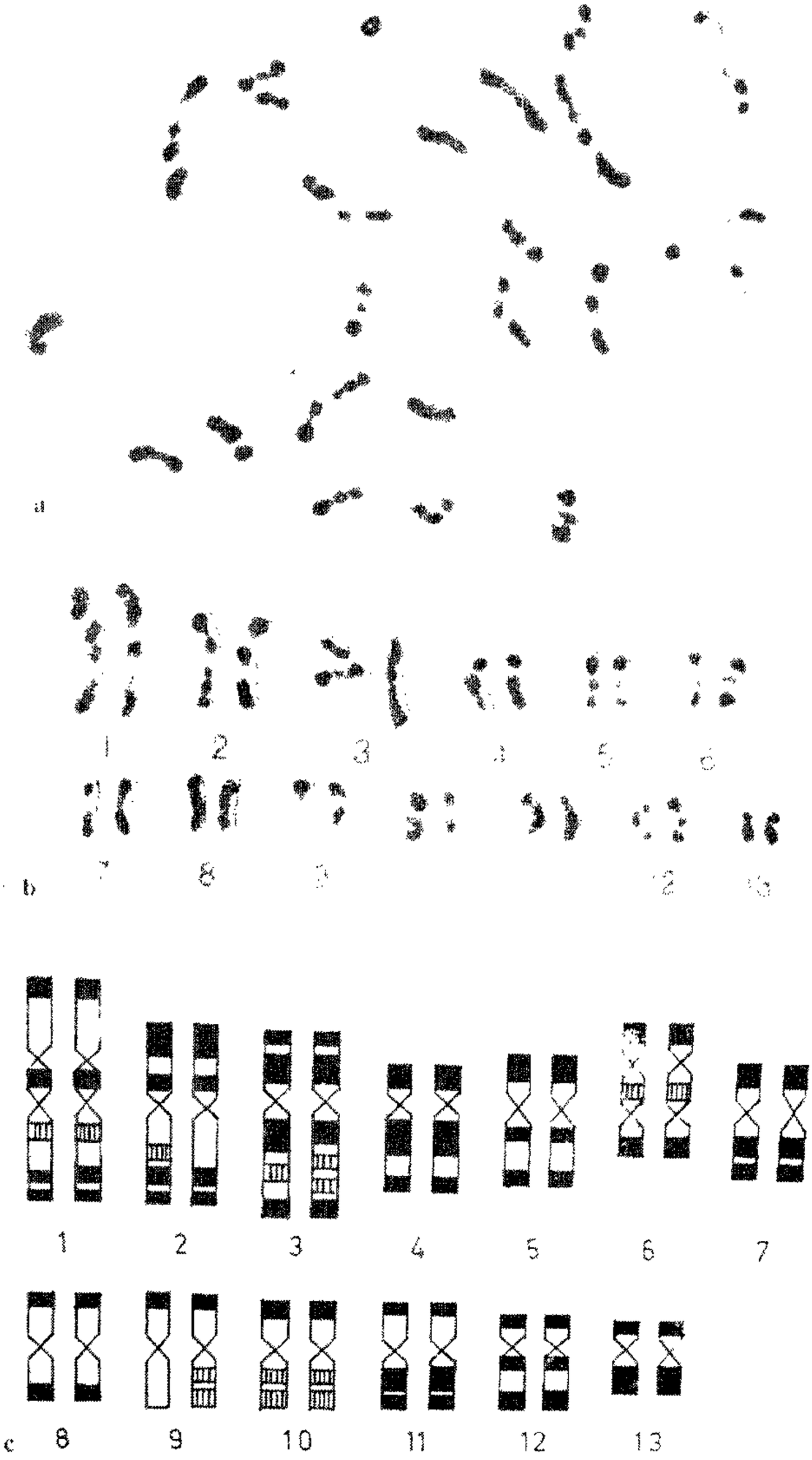

Figure 1. Orcein-banding pattern in chromosomes of *Coccultus villosus* DC. *a*, Metaphase plate (×4600). *b*, Karyotype with orcein-banding pattern (×4600). *c*, Idiogram of *Coccultus villosus* and O-banding pattern. ■ Intensely stained regions, ▥ Intermediately stained regions, □ Pale stained regions.