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Identification of Chromosomes with Secondary Constrictions in *Melilotus* Species

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ABSTRACT: Secondary constrictions were determined in one chromosome pair in the complements of *M. infesta*, *M. macrocarpa*, *M. italica* (subgenus *Micromelilotus*), and *M. alba* and *M. officinalis* (subgenus *Melilotus*). The karyotypes of *M. infesta* and *M. macrocarpa* were found to be similar. Chromosomes of *M. italica* were larger than the chromosomes of the other four *Melilotus* species. The chromosome size in *M. italica* suggests the presence of large chromosomes in an ancestral or pro-*Melilotus* prototype. The chromosomes with satellites of *M. infesta* and *M. macrocarpa* appear to differ in morphology from the satellite chromosomes of *M. alba* and *M. officinalis* by a paracentric inversion. The morphology of the satellite chromosomes in *M. italica* is thought to represent a more primitive type than in the other *Melilotus* species studied.

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THE SWEETCLOVER GENUS, *Melilotus* Adans., contains forage and nitrogen fixing species of agricultural importance. Taxonomically, *Melilotus* is divided into two subgenera[10], (*Eu*)*Melilotus* Schulz and *Micromelilotus* Schulz. Isely[4] considers the subgenus *Melilotus* to consist of nine biennial species, including the commercially important *M. alba* Desr. and *M. officinalis* (L.) Lam., while the subgenus *Micromelilotus* contains 11 annual species, few of which have economic value as crops[14]. Other taxonomists, most notably Suvorov[16], consider the genus to contain a greater number of species.

The chromosome number of many *Melilotus* species, $2n = 2x = 16$, was determined early in this century[2,11]. Karyotype analyses of all *Melilotus* species, with the exception of the dubious *M. bicolor* Boiss & Bal., were reported by Kita[5,6]. Chromosomes with satellites were found in all species except *M. infesta* Guss., *M. macrocarpa* Goss. et Dur., and *M. italica* (L.) Lam., which belong to *Micromelilotus*. Secondary constrictions could not be defined in the chromosomes of these three species.

The present study was undertaken to delineate the occurrence and positions of secondary constrictions in the chromosome complements of *M. infesta*, *M. macrocarpa*, and *M. italica*. Additionally, a comparison was made between the satellite chromosomes of these species and the satellite chromosomes of *M. alba* and *M. officinalis* for evidence of karyotypic evolution.

Materials and Methods

Seed of each species was obtained from the collection of *Melilotus* germplasm that is kept in cold storage at the University of Nebraska. *M. infesta* (Bdn. 61-98) and *M. macrocarpa* (Bdn. 61-97) were from seed of Algerian accessions. Seed of *M. italica* (Bdn. 523) came from a collection made in Portugal. Seed of commercially available cultivars of *M. alba* (Evergreen-Lot 14C) and *M. officinalis* (Yukon-F.C. 40594) were used in the study.

Actively growing root tips from one-month-old seedlings grown in clay pots were utilized. The root tips were placed in ice water for 24 hours (for a detailed description, see Singh and Tsuchiya[12]) and then fixed in a 3:1 mixture of ethanol:propionic acid in which a small amount of ferric chloride had been dissolved as a mordant. After 3–4 days in a fixative, the root tips were hydrolyzed in 1N HCl at 60°C for 6 minutes and stained in acetocarmine for at least 3 days. After sufficient staining, the meristematic tissue was squashed

in 45 percent acetic acid. Dividing cells in the meristematic region were isolated and analyzed.

For the *Micromelilotus* species, the karyological interpretations were made on the chromosome complement of a representative cell from each species in which the chromosomes were well spread and the secondary constrictions were obvious. From these cells, karyotypes were constructed by arranging the chromosomes in pairs considering the total length, centromere position, and gross morphology. The chromosome pairs were generally arranged in gradation from the longest to the shortest pair of the complement. Satellite chromosome morphology in *M. alba* and *M. officinalis* cells was studied in a number of preparations.

Results

The chromosome number of $2n = 2x = 16$, as previously reported[2,5,11], was confirmed in all species. Numerous cells were observed (ca. 20) in each species in which chromosome morphology was able to be characterized. Secondary constrictions in one chromosome pair were found to exist in *M. infesta*, *M. macrocarpa*, and *M. italica* (Figures 1, 2, 3, and 4*A*-*C*). The existence of satellite chromosomes in *M. alba*[3] and *M. officinalis*[1] also was reaffirmed (Figure 4*D* and *E*).

The chromosome complement of *M. infesta* is shown in Figure 1*A*. A secondary constriction was found in the largest chromosome pair near the centromere in the short arm (Figures 1*B* and 4*A*). At somatic prophase, this chromosome pair appeared to be heterochromatic in contrast to the majority of other chromosomes that appeared to stain normally (Figure 5). The karyotype shows the presence of many nonmetacentric chromosomes (Figure 1*B*).

Figure 2*A* shows the somatic chromosomes of *M. macrocarpa*. The chromosomes with satellites in this species were the largest chromosomes in the complement (Figure 2*B*) and appear to be heterochromatic, as in *M. infesta*. In general, the karyotype of *M. macrocarpa* closely resembles the *M. infesta* karyotype. However, the short arm bearing the satellite body in *M. macrocarpa* was shorter, relatively, than the corresponding short arm in *M. infesta* (Figure 4*A* and *B*). The chromosomes of *M. macrocarpa*, excluding the satellite chromosome pair, did not stain as deeply as chromosomes of the other species.

Overall, the chromosomes of *M. italica* were the largest of the species studied (Figure 3*A*). The satellite bodies in this species were the same or of greater length than the long arms of the satellite chromosomes (Figures 3*B* and 4*C*). The larger chromosomes in the karyotype were metacentric while the smaller chromosomes tended to have nonmedian centromeres (Figure 3*B*). The chromosomes of *M. italica* stained very deeply, suggesting a heterochromatic nature.

The satellite chromosomes of *M. alba* and *M. officinalis* are different in morphology from the preceding species (Figure 4*A*-*E*). Both species have small satellite bodi tached to the short arms (Figure 4*D* *E*).

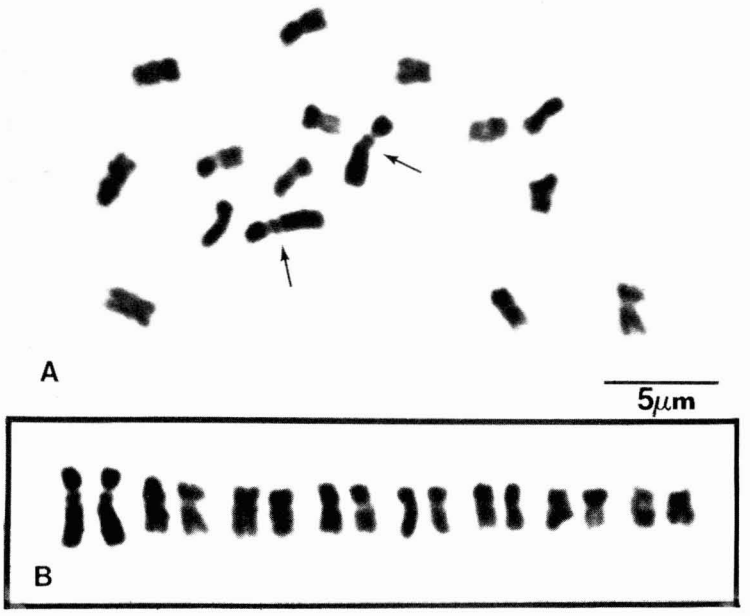

FIGURE 1 *A* shows somatic chromosomes of *Melilotus infesta*, $2n = 2x = 16$; arrows indicate satellite chromosomes. *B*—karyotype of *M. infesta;* chromosomes cut from *A*.

Discussion

Comparisons of the karyotypes of *M* *festa*, *M. macrocarpa*, and *M. italic* served in the present study with those

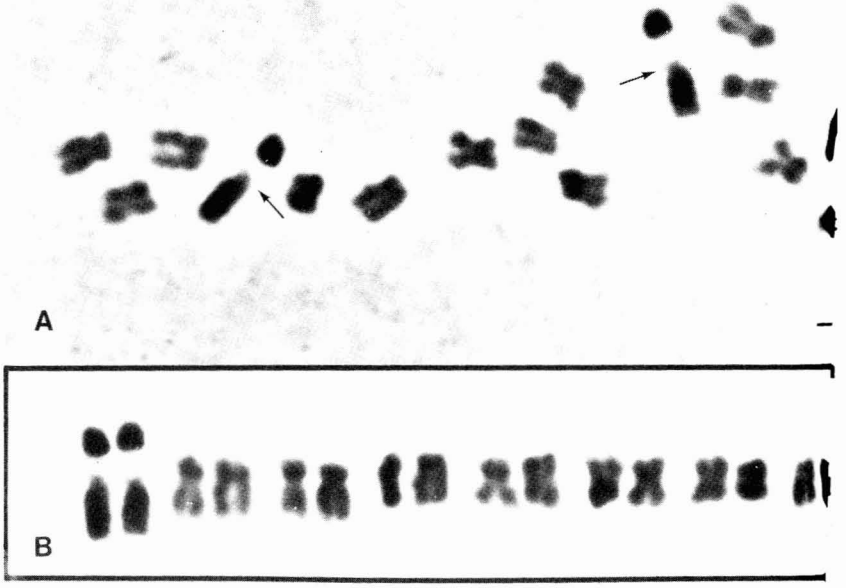

FIGURE 2 *A* shows somatic chromosomes of *Melilotus macrocarpa*, $2n = 2x = 16$; arrows satellite chromosomes. *B*—karyotype of *M. macrocarpa;* chromosomes cut from *A*.

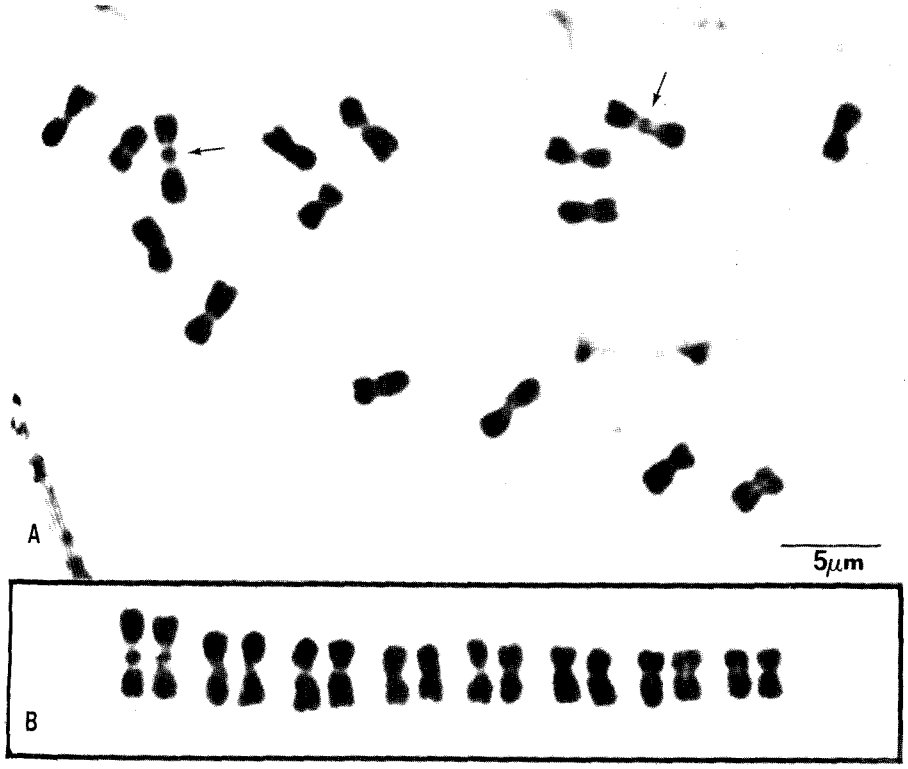

FIGURE 3 *A* shows somatic chromosomes of *Melilotus italica*, $2n = 2x = 16$; arrows indicate satellite chromosomes. *B*—karyotype of *M. italica;* chromosomes cut from *A*.

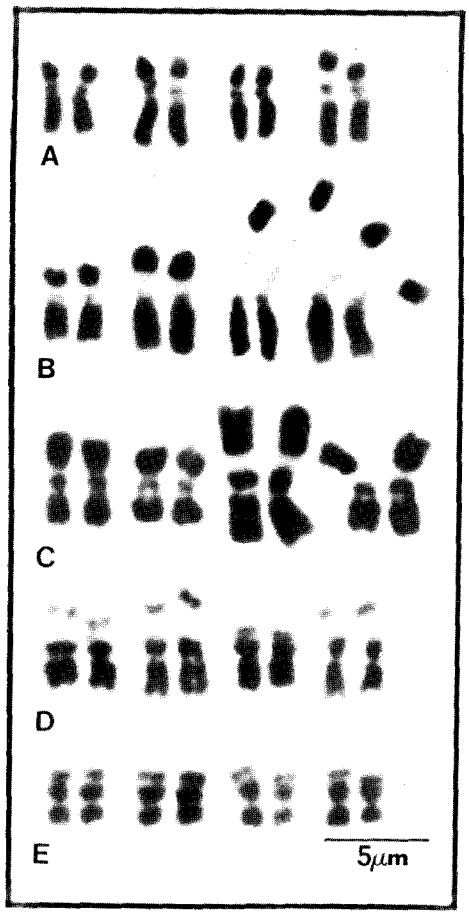

FIGURE 4 Satellite chromosomes of *Melilotus* species isolated from different somatic cells. *A—M. infesta; B—M. macrocarpa; C—M. italica; D—M. alba; E—M. officinalis.*

sented by Kita[6] show a general agreement with the exception of the satellite chromosomes. In each species, it appears that Kita misidentified the secondary constriction as the primary constriction. The small size of the short arm of the satellite chromosomes in *M. infesta* and *M. macrocarpa* causes a close proximity between the primary and secondary constrictions making distinction very difficult (Figure 4*A* and *B*). Also, excessive chromosome contraction and deep staining often obscured the primary constriction in many cells. In the case of *M. italica*, overstaining of the presumably heterochromatic satellite chromosomes probably confounded Kita's observations.

The chromosomes of *M. macrocarpa* are similar to those of *M. infesta*, yet appear substantially larger. This is in contrast with the results of Kita[5,6] who observed no significant difference in chromosome size between the two species. Comparison of the chromosomes of *M. infesta* and *M. macrocarpa* by chromosome index (short arm/long arm ratio) and relative length, following the methodology of Tjio and Hagberg[17], reveals no critical difference between the karyotypes. The size differential found in the present study is probably due to differences in chromosome contraction between the two cells shown of each species and to artifacts normally encountered with the squash technique for slide preparations.

The differences in size between chromosomes of *M. italica* and those of other *Melilotus* species have been reported previously[1,5]. In many plant species, it has been observed that the evolution of karyotypes proceeds from large chromosomes in primitive species to smaller chromosomes in specialized species[15]. Chromosome studies in two leguminous genera, *Trifolium*[9] and *Medicago* (Schlarbaum, unpub.), indicate karyotypic evolution has occurred in this manner. This suggests that the chromosome size of *M. italica* may be indicative of the chromosome size in an ancestral *Melilotus* or pro-*Melilotus* species. This hypothesis is supported by the morphological observations of Suvorov[16], who considered *M. italica* to characterize the features of an ancestral *Melilotus* type, although he recognized that paleobotanic information was lacking for the genus. There is some doubt that the present karyotype of *M. italica* should be considered as a basikaryotype[13] for a *Melilotus* prototype, as it is semi-asymmetrical, indicating an advanced evolutionary state[7,15]. The functional basis of the large size and heterochromatic nature of the *M. italica* chromosomes is not known.

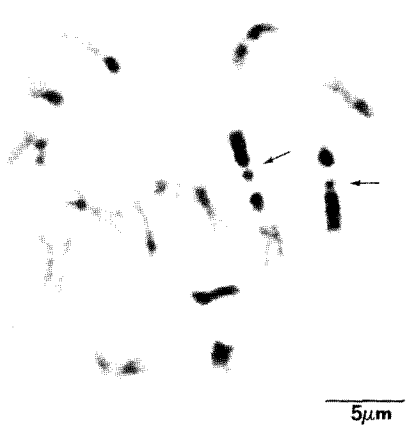

FIGURE 5 Chromosomes of *Melilotus infesta* at somatic prophase; arrows indicate heterochromatic satellite chromosomes.

The satellite chromosomes of the species studied can be grouped into three types: type I—satellite large, short arm large as in *M. italica* (Figure 4*C*); type II—satellite large,

short arm small as in *M. infesta* and *M. macrocarpa* (Figure 4*A* and *B*), and type III—satellite small, short arm large as in *M. alba* and *M. officinalis* (Figure 4*D* and *E*). The differences between types II and III could result from a paracentric inversion with breakage sites in the short arm and satellite body. The satellite chromosome morphology of type I possibly represents a more primitive state than satellite chromosomes of types II and III.

After observing alteration of the nucleolus organizer chromosome system due to irradiation in *Trillium kamtschaticum* Pall., Matsuura[8] proposed that one method of satellite chromosome origin was a paracentric inversion of a terminally located nucleolus organizer region (NOR) to an intercalary position. If the origin of satellite chromosomes in *Melilotus* proceeded according to this hypothesis, the morphology of the satellite chromosomes in *M. italica* (type I) appears similar to what would be expected from a paracentric inversion in a chromosome with a terminal NOR present in a primitive *Melilotus* karyotype.

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