Brazilian Journal of Genetics, 20, 2, 193-196 (1997)
(Revista Brasileira de Genética)

Chromosomal multiformity in *Botanochara bonariensis* (Coleoptera, Chrysomelidae, Cassidinae)

C.E. Stolar[1] and C.J. Bidau[1,2]

ABSTRACT

The Cassidinae are characterized by highly conservative karyotypes, except in the Stolaini. This tribe shows the highest chromosome numbers in Coleoptera and complex chromosomal sex determination systems. We analyzed four natural populations of *Botanochara bonariensis* from Misiones and Entre Ríos Provinces (Argentina). The Concepción del Uruguay sample showed 2n = 47 (male) and 2n = 48 (female), the male meioformula being 22 II + $X_p neo X neoy_p$. The Itacurubí population showed 2n = 27 (male) and 2n = 26 (female), with a male meioformula of 12 II + $neoX_p neoy\ y_p$. The Mbororé and Ñu Porá populations showed 2n = 41 (male) and 2n = 40 (female) (the chromosome number of both samples is the modal one due to the existence of Robertsonian polymorphisms) and the male meioformula is 19 II + $neoX_p neoy\ y_p$. Autosomal morphology also showed wide variation among samples. Our results suggest that chromosomal speciation has been a relevant mechanism in the evolution of the genus *Botanochara*, this process was probably favored by the small size of demes, low vagility and host plant specificity. A taxonomic revision of *B. bonariensis* is needed to clarify the status of this morphospecies, possibly an array of several sibling species.

INTRODUCTION

Coleoptera is a very ancient insect order that comprises the largest number of described species of living organisms (Crowson, 1981). It is characterized by an impressive karyotypic variation at the autosomal and sex chromosome level (White, 1973; Smith and Virkki, 1978; Virkki, 1984). With respect to the latter, beetles show unique systems such as the Xy_p, and complex ones derived from the former through chromosomal rearrangements (Smith and Virkki, 1978).

Classical taxonomy is mainly based on exomorphological characters and most of the South American Coleopteran species have been described under these criteria. These include forms with wide geographical distribution where populations separated by hundreds or thousands of kilometers have been ascribed to a single specific entity, a procedure which in many cases is, at least, dubious. The chrysomelid *Botanochara bonariensis* is probably one of such cases. The study of chromosomes is a useful tool for unravelling such situations since speciation is accompanied in most cases by chromosomal repatterning (White, 1973, 1978; King, 1993).

Chrysomelidae comprises the largest number of known Coleopteran species and is characterized by extensive karyotypic variation. Within the family, the Cassidinae, and especially the Stolaini, represent a clear-cut case of chromosomal differentiation. The genus *Botanochara* has been the subject of chromosomal studies which have demonstrated its notable karyotypic diversity (Vaio and Postiglioni, 1974; Panzera *et al.*, 1983).

B. bonariensis is a morphospecies widely distributed in Argentina, mainly in the Mesopotamic

[1] Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Félix de Azara 1552, (3300) Posadas, Misiones, Argentina. Send correspondence to C.E.S.
[2] CONICET (National Research Council).

region. It has low vagility and high host constancy (Convolvulaceae). These characteristics make of *B. bonariensis* a good candidate for primary chromosomal allopatric divergence processes.

We analyzed the chromosomes of four Argentinian populations of *B. bonariensis* which proved to be karyotypically distinct.

MATERIAL AND METHODS

Adult males of *B. bonariensis* were analyzed. Individuals were captured in coastal zones of the Paraná and Uruguay rivers on the Convolvulaceae *Ipomoea purpurea* and *I. grandifolia*. Four Argentinian populations were sampled: a) Itacurubí (Departamento Capital, Misiones), six males; b) Mbororé (Departamento Capital, Misiones), 12 males; c) Ñu Porá (Departamento Garupá, Misiones), six males, and d) Concepción del Uruguay (Departamento C. del Uruguay, Entre Ríos) eight males.

Testes were dissected out in insect saline and subjected to hypotonic shock in 0.075 M KCl for 15-30 min. They were then fixed in 3:1 (methanol-glacial acetic acid). Some old fixations were processed according to a modified version of Rozek's (1994) technique. Observations were made on standard orcein or hematoxylin preparations.

RESULTS

Chromosomes were analyzed in M I and M II cells and, when possible, in spermatogonial mitoses. Specimens from the Concepción del Uruguay population showed 2n = 47 in the males and 2n = 48 in the females, with 34 telocentric and 10 metacentric autosomes (Figures 1d; 2a). The sex system is of the $X_p neo X$ $neoy_p$ type. The meioformula is 22 II + $X_p neo X neoy_p$ and FN = 54. Analysis of mitotic metaphases in this sample confirmed the meiotic observations (Figure 1a).

The Itacurubí population showed 2n = 27 males and 2n = 26 females, with 24 metacentric-submetacentric chromosomes (Figures 1c;2b). The sex system is of the $neoX_p neoy\ y_p$ type. Thus, the meioformula is 12 II + $neoX_p neoy\ y_p$.

The Mbororé and Ñu Porá samples showed a karyotype of 2n = 41 (this chromosome number of both

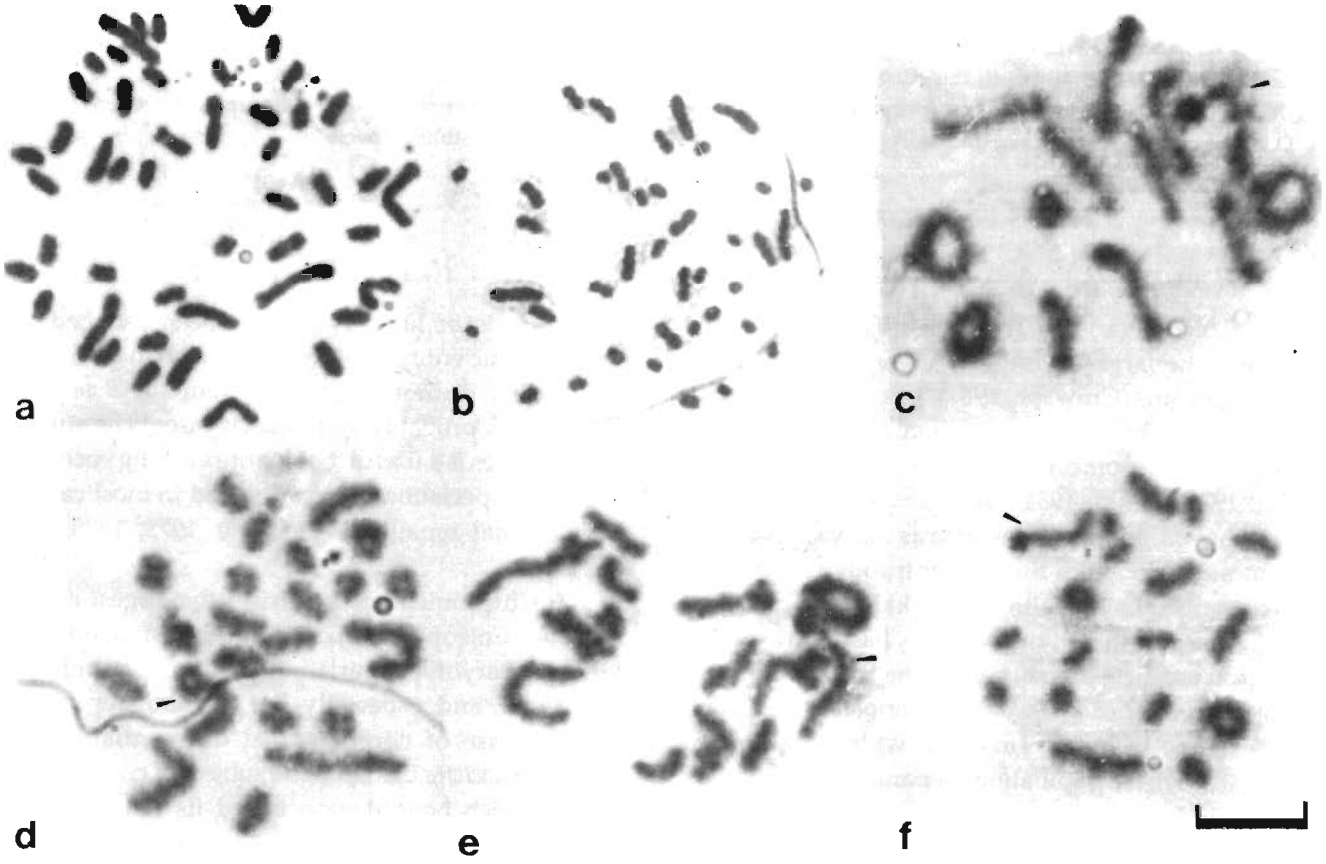

Figure 1 - a-f, *Botanochara bonariensis*. a) Spermatogonial metaphase of a male from Concepción del Uruguay (2n = 47); b) Spermatogonial metaphase of a male from Ñu Porá (2n = 39). c-f), Diakinesis. c) Itacurubí (n = 12 II + $neoX_p neoy\ y_p$); d) Concepción del Uruguay (n = 22 II + $X_p neoX neo\ y_p$); e) Mbororé (n = 16 II + 2 III + $neoX_p neoy\ y_p$); f) Ñu Porá (n = 17 II + $neoX_p neoy\ y_p$). In c, d, e and f an arrowhead marks the sex trivalent. Bar = 10 µm.

populations is the modal one since these entities exhibited a very complex Robertsonian system which is at present under study), with 24 telocentric and 14 biarmed autosomes. The sex system is of the $neoX_pneoy\ y_p$ type, and the modal meioformula is thus 19 II + $neoX_pneoy\ y_p$ (FN = 52) (Figures 1b,e,f; 2c,d).

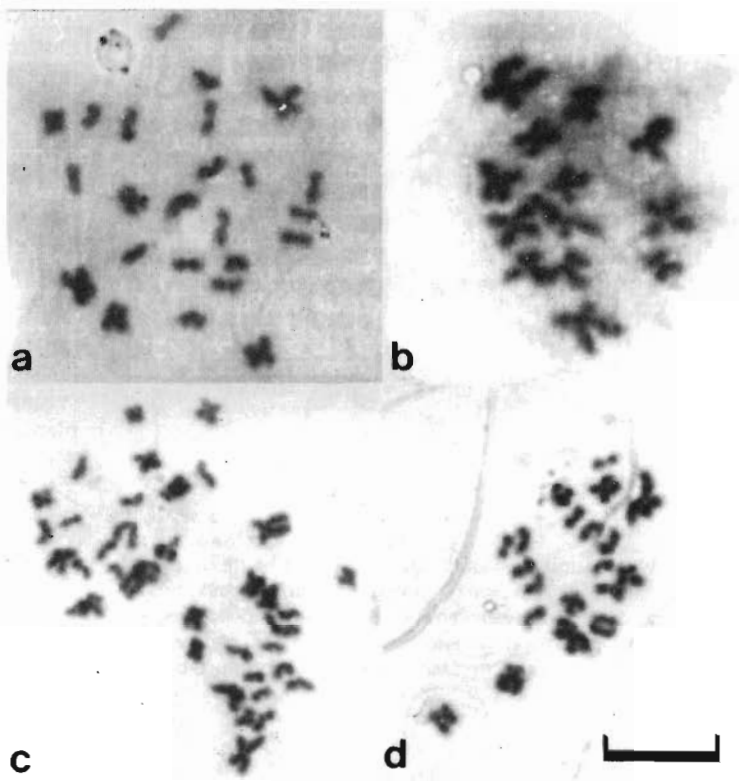

Figure 2 - a-d, *Botanochara bonariensis*. Metaphase II. a) Concepción del Uruguay (n = 24); b) Itacurubí (n = 13); c) Mbororé, two sister cells (n = 19, n = 18); d) Ñu Porá (n = 20). Bar = 10 µm.

DISCUSSION AND CONCLUSIONS

All populations of *Botanochara bonariensis* analyzed in the present work showed differences in chromosome number, morphology, fundamental number and chromosomal sex determination system. They also proved to be chromosomally distinct from other species of the genus (Vaio and Postiglioni, 1974; Panzera *et al.*, 1983). Furthermore, no population studied by us shared the chromosomal features described by Panzera *et al.* (1983) for the same species from Uruguay (Table I).

These remarkable differences are probably due to multiple chromosomal rearrangements. Multiple chromosomal sex-determination systems, present in all the analyzed populations, have probably originated by incorporation of autosomes into a standard Xy_p sex system through translocations (Stolar, 1995).

In the Itacurubí, Mbororé and Ñu Porá populations, the multiple sex system was produced by the fusion between X_p and a medium sized subtelocentric autosome. Furthermore, the sex trivalent included a medium sized telocentric *neoy*, a minute y_p and a medium sized $neoX_p$ (see Figure 3 and Stolar, 1995). The sex trivalent in the specimens from Concepción del Uruguay was originated by an interchange between the minute y_p and a subtelocentric autosome (Figure 3 and Stolar, 1995).

The Cassidinae are characterized by an 8 II + Xy_p meioformula. However, the Stolaini and especially the genus *Botanochara* show high chromosome numbers and complex chromosomal sex determination systems (Petitpierre *et al.*, 1988) (Table I).

The chromosomal rearrangements that these populations underwent were probably favored by the small size of their demes and low vagilities, determined

Table I - List of species of the genus *Botanochara* analyzed to date.

Species	2n (Male)	Meioformulae (Male)	Authors
Botanochara angulata	51	24 II + $X_pneoXneoy_p$	Vaio and Postiglioni (1974)
B. bonariensis	44	20 II + $X^1_p\ X^2_p\ neoXneo\ y_p$	Panzera *et al.* (1983)
B. duodecimverrucata	44	20 II + $X^1_p\ X^2_p\ neoXneo\ y_p$	Panzera *et al.* (1983)
Botanochara sp.	44	20 II + $X^1_p\ X^2_p\ neoXneo\ y_p$	Panzera *et al.* (1983)
B. bonariensis (C. del Uruguay)	47	22 II + $X_pneoXneo\ y_p$	Stolar and Bidau (this paper)
B. bonariensis (Itacurubí)	27	12 II + $neoX_pneoy\ y_p$	Stolar and Bidau (this paper)
B. bonariensis (Mbororé)	41	19 II + $neoX_pneoy\ y_p$	Stolar and Bidau (this paper)
B. bonariensis (Ñu Porá)	41	19 II + $neoX_pneoy\ y_p$	Stolar and Bidau (this paper)

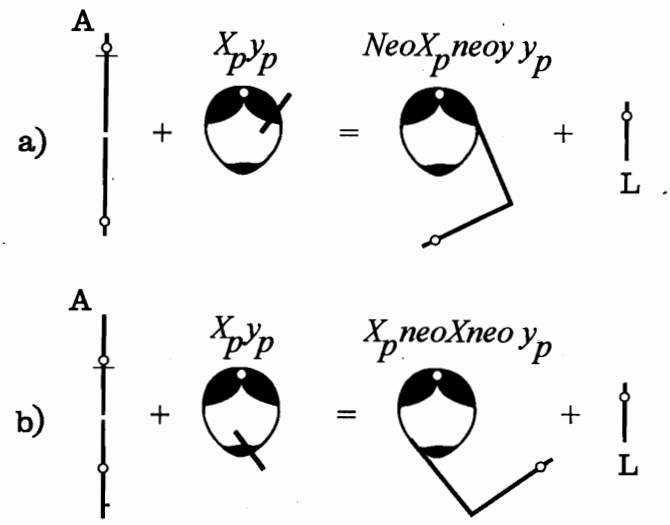

Figure 3 - Hypothetical mechanism for the origin of the multiple sex-chromosomes of *Botanochara bonariensis* from an Xy_p ancestor. a) Reciprocal translocation between an acrocentric autosome and the X_p chromosome; b) reciprocal translocation between a metacentric autosome and the y_p. In both cases, the second product of the rearrangement is lost (L).

by their limited dispersion ability by flight and the specifity of diet and host plants (Petitpierre, 1987).

Although the Mbororé and Itacurubí forms are sympatric in at least one locality (Itacurubí), no evidence of hybridization was found, suggesting at least incipient reproductive isolation. Nevertheless, no clear morphometric differences were found between them (Stolar, 1995), reinforcing the hypothesis that *B. bonariensis* is in fact a complex of several synmorphic species whose main mode of speciation has been chromosomal.

ACKNOWLEDGMENTS

We wish to thank Lic. Mabel D. Giménez and Mr. Esteban Braggio for constructive criticism, valuable suggestions and help in the collection of material. We are also grateful to Dr. Axel O. Bachmann for help in the identification of the specimens. The continuous support of CONICET is gratefully acknowledged.

RESUMO

Cassidinae são caracterizados por cariótipos altamente conservadores, exceto na Stolaini. Esta raça apresenta os números mais elevados de cromossomos nos Coleopteros e complexos sistemas cromossômicos determinantes do sexo. Nós analisamos quatro populações naturais de *Botanochara bonariensis* das províncias de Misiones e Entre Ríos (Argentina). A amostra de Concepción del Uruguai mostrou 2n = 47 (macho) e 2n = 48 (fêmea), sendo a meiofórmula para o macho 22 II + $X_p neoXneo\ y_p$. A população de Itacurubí mostrou 2n = 27 (macho) e 2n = 26 (fêmea), com uma meiofórmula para o macho de 12 II + $neoX_p neoy\ y_p$. As populações de Mbororé e Ñu Porá mostraram 2n = 41 (macho) e 2n = 40 (fêmea) (o número de cromossomos de ambas amostras é o modal devido a existência de polimorfismos Robertsonianos) e a meiofórmula para o macho é 19 II + $neoX_p neoy\ y_p$. A morfologia cromossômica também mostrou ampla variação entre as amostras. Nossos resultados sugerem que a formação das variedades cromossômicas foi um mecanismo relevante na evolução do gênero *Botanochara*, este processo foi provavelmente favorecido pelo pequeno tamanho das colônias dos demes, baixa vagilidade e especificidade para planta hospedeira. Uma revisão taxonômica do *B. bonariensis* é necessária para esclarecer o status desta morfoespécie, possivelmente um arranjo de várias espécies parentes.

REFERENCES

Crowson, R.A. (1981). *The Biology of the Coleoptera*. Academic Press, London, New York.

King, M. (1993). *Species Evolution. The Role of Chromosome Change*. Cambridge University Press, Cambridge.

Panzera, F., Mazzella, M.C. and **Vaio, E.S. de** (1983). Cytological studies on three species of neotropical cassidines (Coleoptera, Chrysomelidae). *Genetica 62*: 61-68.

Petitpierre, E. (1987). Why beetles have strikingly different rates of chromosomal evolution? *Elytron 1*: 25-32.

Petitpierre, E., Segarra, C., Yadav, J.S. and **Virkki, N.** (1988). Chromosome numbers and meioformulae of Chrysomelidae. In: *Biology of Chrysomelidae* (Jolivet, P., Petitpierre, E. and Hsiao, T.H., eds.). Klüwer, Dordretch, pp. 161-168.

Rozek, M. (1994). A new chromosome preparation technique for Coleoptera (Insecta). *Chromosome Res. 2*: 76-78.

Smith, S.G. and **Virkki, N.** (1978). Coleoptera. In: *Animal Cytogenetics 3. Insecta 1* (John, B., ed.). Gebrüder Borntraeger, Berlin-Stuttgart.

Stolar, C.E. (1995). Citogenética y citotaxonomía de *Botanochara bonariensis* (Coleoptera, Chrysomelidae, Cassidinae). Tesis de Licenciatura. Universidad Nacional de Misiones.

Vaio, E.S. de and **Postiglioni, A.** (1974). Stolaine cassidines (Coleoptera, Chrysomelidae) with Xy_p sex chromosomes and derivative system X_p neoX neoy_p. *Can. J. Genet. Cytol. 16*: 433-440.

Virkki, N. (1984). Chromosomes in evolution of Coleoptera. In: *Chromosomes in Evolution of Eukaryotyc Groups. Vol. II* (Sharma, A.K. and Sharma, A., eds.). CRC Press, Boca Raton, pp. 41-76.

White, M.J.D. (1973). *Animal Cytology and Evolution*. 3rd edn. Cambridge University Press, London.

White, M.J.D. (1978). *Modes of Speciation*. W.H. Freeman, San Francisco.

(Received July 29, 1996)