Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Clara Goday, Anna Paola Bianchi Bullini,
Giuseppe Nascetti, Luciano Bullini

Chromosome studies on Bacillus atticus, B. rossius and their hybrids (Cheleutoptera, Bacillidae)

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **71** (1981), n.5, p. 126–133.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1981_8_71_5_126_0>

Genetica. — *Chromosome studies on* Bacillus atticus, B. rossius *and their hybrids* (Cheleutoptera, Bacillidae) (*). Nota di CLARA GODAY (**), ANNA PAOLA BIANCHI BULLINI (**), GIUSEPPE NASCETTI (**) e LUCIANO BULLINI (**), presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — Viene descritto il corredo cromosomico del fasmide *Bacillus atticus*, specie a partenogenesi telitoca diffusa in Grecia e Iugoslavia, recentemente scoperta in Italia meridionale (*B. atticus caprai*). Esso è costituito da 34 elementi, di cui 4 di grandi dimensioni, 2 metacentrici e 2 submetacentrici. Le 17 coppie sono spesso eteromorfe, ciò che suggerisce un'origine ibrida di questa specie. Il confronto con il cariotipo di *B. rossius* ($2n = 36$ nella femmina) mostra differenze non solo nel numero dei cromosomi (due piccoli telocentrici in più) ma anche nella loro morfologia. In particolare, la coppia n. 2 è submetacentrica in *B. atticus* e telocentrica in *B. rossius*. Il numero fondamentale è lo stesso nelle due specie e una fusione centrica in *B. rossius* tra la piccola coppia telocentrica (assente in *B. atticus*) e la grande coppia telocentrica (n. 2) darebbe origine a un cariotipo simile a quello di *B. atticus* sia nel numero cromosomico che nella morfologia della coppia n. 2. Lo studio degli ibridi tra le due specie (ottenibili in laboratorio ma presenti anche in natura) ha mostrato, come atteso, un numero cromosomico di 35 nella femmina (18 cromosomi derivanti da *B. rossius* e 17 da *B. atticus*) e di 34 nel maschio (17 cromosomi derivanti da ciascuna delle specie parentali). Non sono state osservate differenze a livello cariologico tra *B. atticus atticus* della Grecia e *B. atticus caprai* della Calabria.

INTRODUCTION

Only three species of the Bacillidae family hawe been investigated up to now at the chromosome level: *Phalces longiscaphus* de Haan, *Bacillus rossius* (Rossi) and *Clonopsis gallica* (Charp.). A chromosome number of $2n = 36$ (35 in the male) was observed in the bisexual *Ph. longiscaphus* (Favrelle [4]; Cappe de Baillon *et al.* [3]) and in the bisexual and parthenogenetic populations of *B. rossius* (Cappe de Baillon *et al.* [2]; Montalenti and Fratini [6]; Mosti and Scali [7]), while $2n = 54 - 56$ was found in the parthenogenetic *Cl. gallica* (Bullini and Bianchi Bullini [1] and unpublished data).

In the present paper the karyotype of a fourth Bacillid species, the parthenogenetic *Bacillus atticus* Brunner, is described and compared to that of the related *B. rossius*. Field and laboratory hybrids between these two species are also investigated.

(*) Research supported by grants of the Consiglio Nazionale delle Ricerche, Rome (Italy) and the Consejo Superior de Investigaciones Científicas, Madrid (Spain).

(**) Institute of Genetics, Faculty of Sciences, University of Rome (Italy).

(***) Nella seduta del 21 novembre 1981.

MATERIALS AND METHODS

Two populations of *B. atticus* were studied: one from Argos, Greece, belonging to the type subspecies and the other from Capo Rizzuto, Calabria, belonging to the Italian subspecies *B. atticus caprai* (Nascetti and Bullini [8]). The *B. rossius* population used for comparison was from Torre Orsaia, Campania. Hybrids between *B. atticus caprai* and *B. rossius* were from Alimini (Apulia) and from laboratory crosses.

The cytogenetic study was performed on metaphases obtained from neural embryonic tissue (neural ganglia) and in the case of adults from follicular epithelium of ovarioles. The methods of preparation of the metaphase chromosomes were those described for *Drosophila* (Gatti *et al.* [5]) with some minor modifications.

The adult females were injected with 0.01 ml of a 2 % colchicine solution for 3 hrs before extracting the ovarioles epithelium. Neural ganglia were incubated in a 10^{-5} M colchicine physiologic solution for 2 hrs. In both cases the tissues were fixed very rapidly after hypotonic treatment (0.0375 M KCl solution for 12 minutes) with a 3 : 1 mixture of acetic acid and methyl alcohol; they were then squashed in 45 % acetic acid under a siliconized coversip that was removed by freezing on dry ice; slides were air dried.

Some slides were stained with Giemsa (4 %, pH 7 for 10 minutes) and others were treated for banding procedures as follows:

H-bands (fluorescence).

Slides were rehydrated for 5 minutes in 0.15 M NaCl—0.03 M KCl—0.01 M Na_2HPO_4 (pH 7), stained for 10 minutes in 0.25 μgr Hoechst 33258 dissolved in the same buffer and after rapid washing were mounted in 0.16 M Na_2HPO_4—0.04 M $Na_3H_5C_6O_7$ (pH 7). Chromosome fluorescence was observed under a Zeiss fluorescence microscope provided with incident illumination using a 200 W mercury light source. The combination of Z×B 612, FT 510, LP 515 filters was used. Once photographed, the slides were destained by immersion in distilled water for 30 minutes.

C-bands

Slides were treated with a saturated barium water solution for 6 minutes, washed in distilled water and then incubated in 2×SSC at 65 °C for 1 hr. They were then thoroughly washed in distilled water and stained with Giemsa (4 %, pH 7) for 20 minutes.

RESULTS

Bacillus atticus *karyotype.*

As shown in Table I, a nearly constant number of 34 chromosomes was found in 54 metaphases from 10 individuals, 5 from Greece (*B. atticus atticus*)

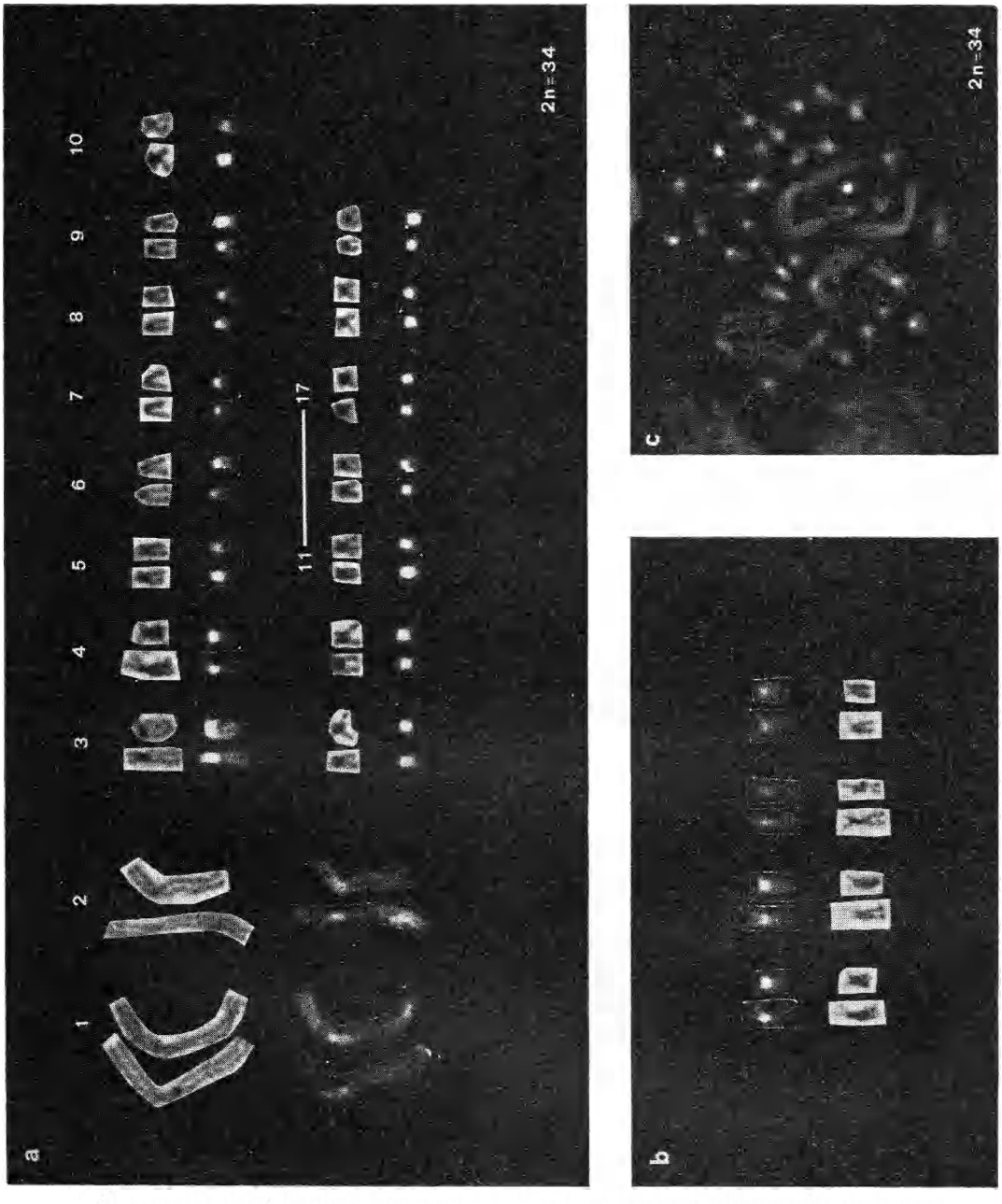

Fig. 1. *a*: *Bacillus atticus* karyotype: C–banding (above) and H-banding (below) of the same metaphase;

b: *B. atticus* pairs no. 4 from different metaphases: H-banding (above) and C-banding (below);

c: *B. atticus* metaphase stained with Hoechst 33258.

and 5 from Southern Italy (*B. atticus caprai*). No differences at the chromosome level were observed between the two subspecies. Among the 17 chromosome pairs, no. 1 is large metacentric, no. 2 large submetacentric, no. 3–5 submetacentric, no. 6–9 telocentric, no. 10 submetacentric, no. 11–17 with a not clearly identifiable centromere position (Fig. 1*a*). Heteromorphism of a number of chromosome pairs is present, easily recognizable in the larger chromosomes (Fig. 1*a*, *b*), apparently indicating a hybrid origin of *B. atticus*.

TABLE I.

Chromosome numbers observed in Bacillus atticus atticus, B. atticus caprai, B. rossius *and their hybrids*

Species	Individuals examined	Metaphases observed	Chromosome number					
			<33	33	34	35	36	>36
B. atticus atticus (♀♀)	5	27	2	4	21	—	—	—
B. atticus caprai (♀♀)	5	27	2	3	20	—	—	—
B. rossius (♀♀)	8	60	1	—	2	5	52	—
Hybrid (♀)	1	32	2	1	2	23	—	4
Hybrid (♂)	1	18	1	2	12	1	2	—

With regard to the fluorescent pattern (Hoechst 33258) we did not obtain any longitudinal differentiation along the chromosomes at the concentration used (Fig. 1). The only fluorescent heterochromatic blocks are the centromeric ones and often this fluorescence is very low or absent especially for the two first pairs of chromosomes.

These results are in agreement with those obtained with C-banding with the only exception of chromosome pair no. 4, where the non-fluorescent long arms are deeply stained (Fig. 1*a*, *b*).

Bacillus rossius *karyotype and comparison with that of* B. atticus.

Confirming previous data from the literature, 36 chromosomes in the female and 35 chromosomes in the male were found in *B. rossius* (Table I). Among the 18 pairs, no. 1 is large metacentric, no. 2 telocentric, no. 3 submetacentric, no. 4 (sexual chromosomes) subtelocentric, no. 5 submetacentric, no. 6–9 telocentric, no. 10 submetacentric; the most probable composition of pairs no. 11–18 is: 2 pairs of telocentric chromosomes, 2 pairs of submetacentric chromosomes and 4 pairs of acrocentric chromosomes (Fig. 2 *a*).

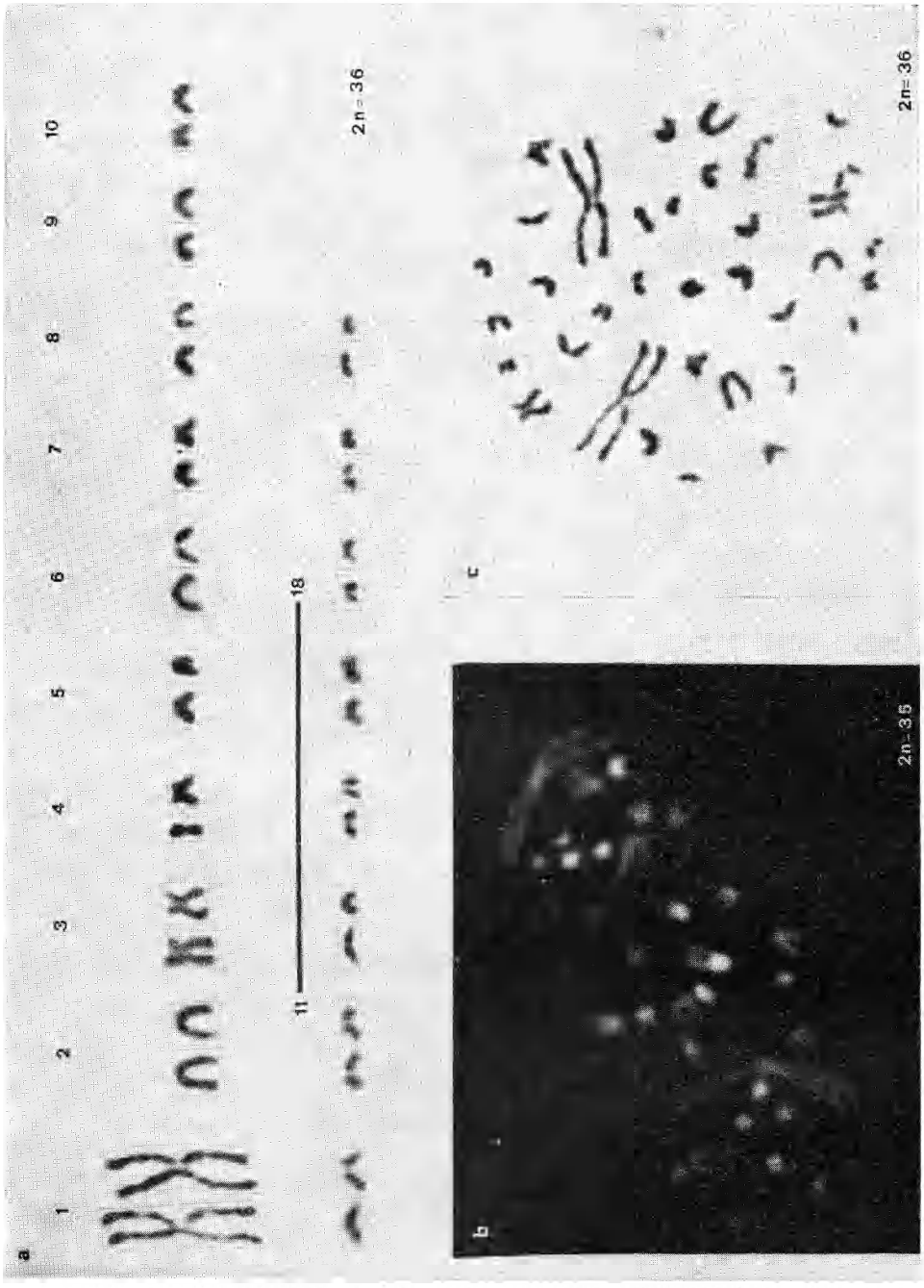

Fig. 2. *a*: *B. rossius* female karyotype stained with Giemsa; *b*: *B. rossius* male metaphase ($2n = 35$) stained with Hoechst 33258; *c*: *B. rossius* female metaphase ($2n = 36$) stained with Giemsa.

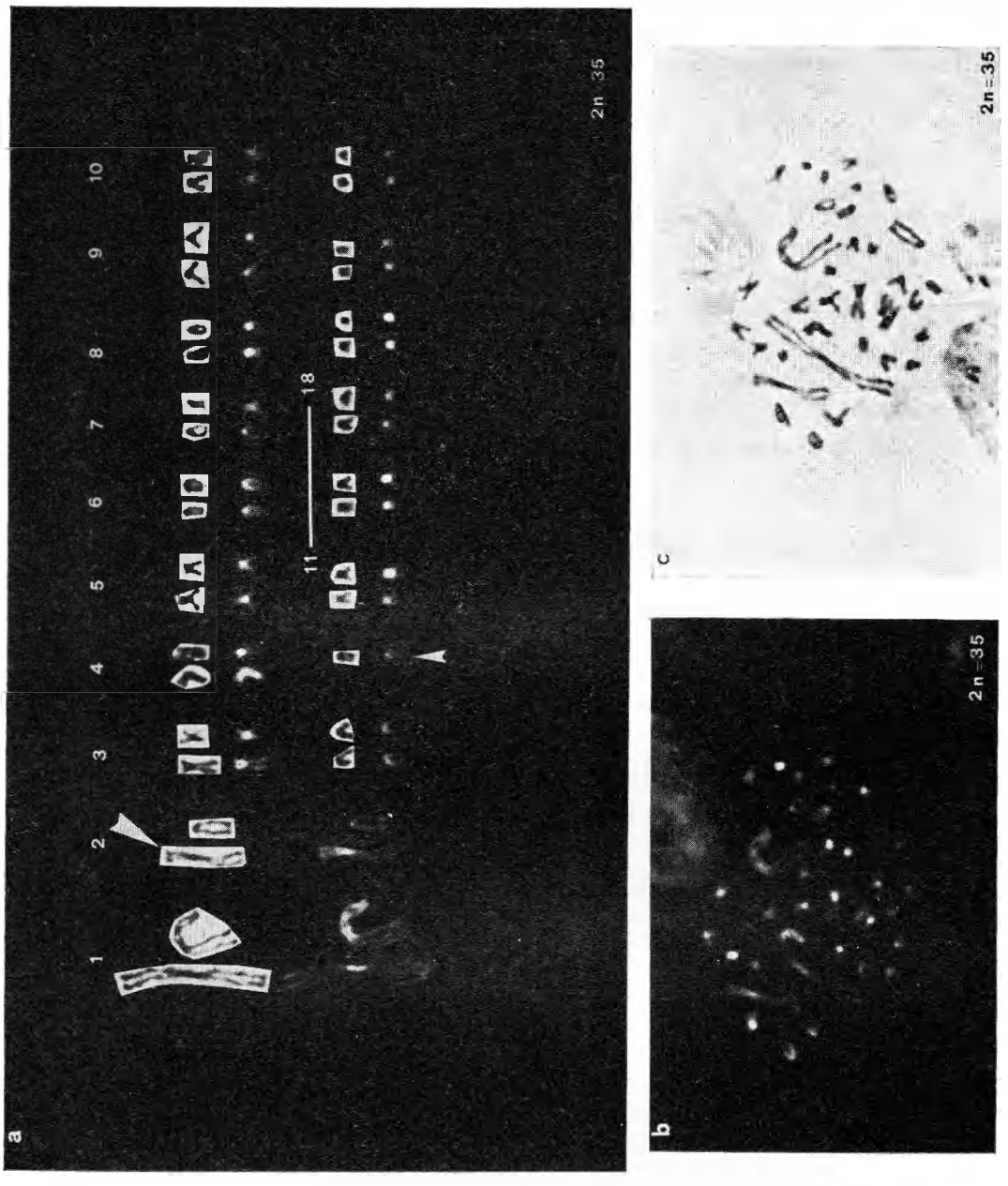

Fig. 3. *a*: Hybrid female karyotype: C-banding (above) and H-banding (below) of the same metaphase; pair no. 2 shows the large submetacentric chromosome from *B. atticus* (big arrow) and the large telocentric one from *B. rossius*; the small arrow indicates the unpaired small *B. rossius* telocentric chromosome; *b*: hybrid female metaphase stained with Hoechst 33258; *c*: the same, C-banded.

As was the case in *B. atticus*, when staining with the fluorochrome Hoechst 33258 we did not find any longitudinal differentiation along the chromosomes at the concentration used (Fig. 2 *b*). Concerning the C–banding, not constantly obtained, our results seem to be the same as for *B. atticus*, i.e.: the staining of the long arm in one chromosome pair (no. 4) besides that of the centromeric region.

Karyotypes of *B. atticus* and *B. rossius* differ mainly: *a*) in chromosome number (36 *versus* 34 due to an extra pair of small telocentric chromosomes in *B. rossius*); *b*) in chromosome pair no. 2, being telocentric in *B. rossius* while submetacentric in *B. atticus*; *c*) due to the presence in the *B. atticus* karyotype of a number of heteromorphic pairs. The fundamental number is the same in the two species. A centric fusion in *B. rossius* between the extra pair of small telocentric chromosomes and the large telocentric pair no. 2 would originate a karyotype similar to that of *B. atticus* in chromosome number and in pair no. 2 morphology.

B. rossius × B. atticus *hybrids karyotype.*

The chromosome number of the hybrid female studied was, as expected, 35 (Table I), with 18 chromosomes from *B. rossius* and 17 from *B. atticus*. This can be easily seen in pair no. 2, the telocentric chromosome being from *B. rossius* and the submetacentric one from *B. atticus* (Fig. 3 *a*). The unpaired small telocentric chromosome is obviously part of the *B. rossius* complement.

As to the hybrid male, the specimen studied showed, as expected, a chromosome number of 34 (Table I) with 17 chromosomes from each parental species.

Acknowledgements. The authors are grateful to Professor Sergio Pimpinelli and Dr. Silvia Buonaccorsi for their helpful collaboration.

References

[1] L. Bullini and A. P. Bianchi Bullini (1971) – *Ricerche sulla riproduzione e sul corredo cromosomico del fasmide* Clonopsis gallica (*Cheleutoptera, Bacillidae*). « Acc. Naz. Lincei, Rend. Cl. Sc. Fis., Mat. e Nat. », *51*, 563–568.

[2] P. Cappe De Baillon, M. Favrelle and G. De Vichet (1937) – *Parthénogenèse et variation chez les Phasmes. III.* Bacillus rossii *Rossi*, Epibacillus lobipes *Luc.*, Phobaeticus sinetyi *Br.* Parasosibia parva *Redt.*, Carausius rotundato–lobatus *Br.* « Bull. Biol. Fr. Belg. », *71*, 129–189.

[3] P. Cappe De Baillon, M. Favrelle and G. De Vichet (1938) – *Parthénogenèse et variation chez les Phasmes. IV. Discussion des faits. – Conclusions.* « Bull. Biol. Fr. Belg. », *72*, 1–47.

[4] M. Favrelle (1935) – *Formule chromosomique et spermatogénèse du* Phalces longiscaphus *de Haan* (*Orthoptère Phasmidae*). « La Cellule », *44*, 175–186.

[5] M. GATTI, S. PIMPINELLI and G. SANTINI (1976) – *Characterization of* Drosophila *heterochromatin. I. Staining and decondensation with Hoechst 33258 and quinacrine.* « Chromosoma », *57*, 377–386.

[6] G. MONTALENTI and L. FRATINI (1959) – *Observations on the spermatogenesis of* Bacillus rossius (*Phasmoidea*). « Proc. XVth Intern. Congr. Zool. London », 749–750.

[7] P. MOSTI and V. SCALI (1975) – *Osservazioni sul corredo cromosomico di* Bacillus rossius, *Insecta Cheleutoptera*. « Acc. Naz. Lincei, Rend. Cl. Sc. Fis., Mat. e Nat. », *59*, 537–545.

[8] G. NASCETTI and L. BULLINI (1982) – *A new phasmid from Italy*: Bacillus atticus caprai (*subsp. n.*) (*Cheleutoptera, Bacillidae*). « Fragmenta Entomologica », *16*, in press.