ELECTRONIC DELIVERY COVER SHEET

Warning Concerning Copyright Restrictions

Cytogenetics and cytotaxonomy of seven Iberian species of *Brachinus* Weber (Coleoptera, Carabidae)

J. Galián, A. S. Ortiz and J. Serrano

Departamento de Biología Animal y Ecología, Facultad de Veterinaria, Universidad de Murcia, 30071, Murcia, Spain

Abstract

The chromosome number of seven species belonging to four subgenera of the genus *Brachinus* was investigated. The presence of a tetravalent in meiosis of *Brachinus longicornis*, a trivalent in some individuals of *B. sclopeta* and a numerical polymorphism in three species, suggests a high karyotypic dynamism within the genus. These data together with those previously reported on *Brachinus* corroborate 2n = 32 as the basic number of the genus and the existence of a repeated trend towards lower numbers within several subgenera.

Introduction

Cytogenetic studies have been carried out on fourteen species of the genus *Brachinus*, mainly of the Palearctic fauna but three involved the Nearctic and Oriental fauna (references in Table 2). The data led Serrano (1982, 1986a) to suggest the hypothesis that 2n = 32 may be the basic number of *Brachinus* and that numerical decreases occur with alternations of the XO-XY systems.

The aim of this work was to describe the karyotypes and meiotic characteristics of seven Iberian species of *Brachinus* and to discuss their cytotaxonomical significance.

Materials and methods

The species analysed were collected in the Iberian peninsula, and the localities and number of individuals are listed in Table 1. Identification was made by the authors. Karyological analyses were carried out on gonads dissected from anaesthetized beetles (ethyl-acetate), treated hypotonically (10—20 min) in either sodium-acetate (0.04 M) or Ohnuki's modified solution (Weber, 1968) plus colchicine 0.05%, fixed with ethanol-acetic acid (3:1), stained with lacto-propionic orcein and squashed. Meiotic metaphases I and II and spermatogonial mitosis were analysed and photographed with a Zeiss phase contrast photomicroscope.

Results

Brachinus (*Brachinus*) *crepitans* has a diploid number of 2n = 39 and an asymmetric karyotype made up of subtelocentric and submetacentric chromosomes, a finding not frequent among carabid beetles. The largest pair appears to be heteromorphic in the two individuals studied. There is an odd metacentric element about the size of pairs 4—5 which may be the X chromosome (Figure 1).

Table 1 Chromosome number of seven Iberian species of *Brachinus*

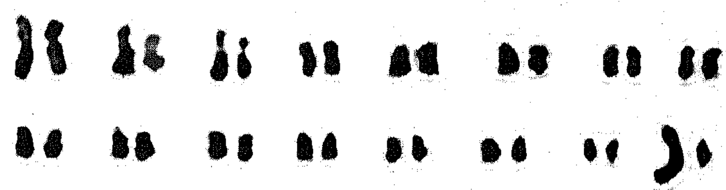
Species and synonyms	2n	n	Localities*	Number of individuals
B. (*Brachinus*) *crepitans* (L., 1758)	39	–	1	2
B. (*Brachinus*) *plagiatus* Reiche, 1868	32	–	2	1
B. (*Brachinolomus*) *longicornis* Fairm., 1858	32	–	3	3
	34	–	3, 4	4
	–	15 + IV	3	2
B. (*Brachinidius*) *bodemeyeri* Apfelb., 1904	25	–	5	2
B. (*Brachinidius*) *sclopeta* (F., 1792)	32	15 + XY	3	3
	31	14 + III	3, 5, 6, 7	8
B. (*Brachinoaptinus*) *baeticus* Ramb., 1842	33	–	8	1
B. (*Brachinoaptinus*) *pateri* Puel, 1838 (*B. oscuratus*, Mateu)	22	–	5	1
	20	9 + XY	5, 9	10

* 1, Embalse del Taibilla, Albacete; 2, Tarifa, Cádiz; 3, El Algar, Murcia; 4, Embalse Alfonso XIII, Murcia; 5, Sierra Espuña, Murcia; 6, Albatera, Alicante; 7, Arroyo del Capitan, Albacete; 8, Sierra Nevada, Granada; and 9, Calar del Mundo, Albacete.

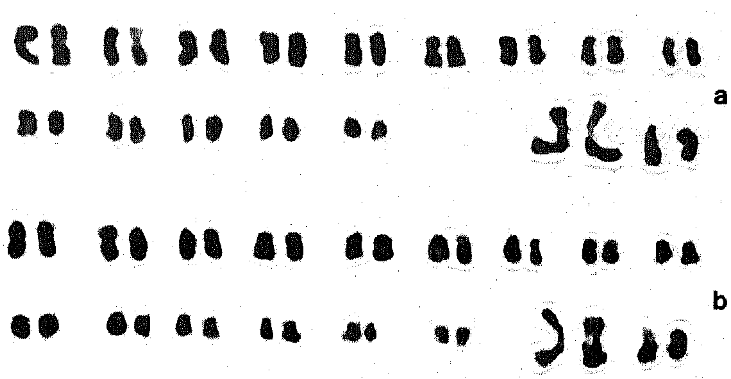
The single male studied of *B.* (*Brachinus*) *plagiatus* has 2n = 32 chromosomes. The karyogram is made up of meta- and submetacentric chromosomes except for the third pair which is subtelocentric. A large submetacentric pair is prominent, and the largest element of the karyogram and one of the smallest ones may be the sex-chromosomes (Figure 2).

B. (*Brachinolomus*) *longicornis* shows two different diploid numbers. Of the five males from El Algar three have 2n = 32 and two have 2n = 34; this latter number also occurs in the two individuals from Embalse Alfonso XIII. These individuals only provided spermatogonial mitoses. Meiotic results were observed in two males from El Algar which show fifteen bivalents and a tetravalent. In tentative karyograms at least three chromosomes have no homologue, both in individuals with 2n = 32 (Figure 3a) and 2n = 34 (Figure 3b). As suggested below these chromosomes may form part of a complex system of sex chromosomes. In metaphase I (Figures 4 and 5) the tetravalent is present in all cells and in most of them there is also a heteromorphic bivalent. An alternate co-orientation of the tetravalent is frequent.

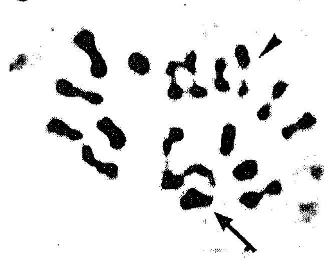
1



2



3



4

5

Figure 1 *Brachinus crepitans*. Karyogram, 2n = 39. x2,000.

Figure 2 *B. plagiatus*. Karyogram, 2n = 32. x2,000.

Figures 3 to **5** *B. longicornis*. Karyograms with 2n = 32 (Figure 3a) and with 2n = 34 (Figure 3b); metaphases I (Figures 4 and 5) with 2n = 34 showing the tetravalent (arrow) and the heteromorphic pair (arrowhead). x2,000.

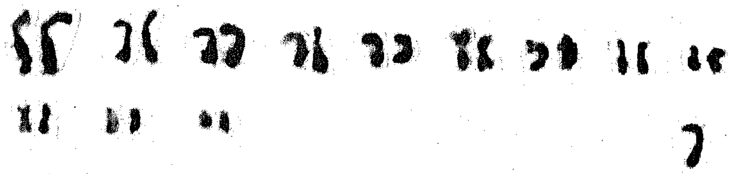
Table 2 Male karyotypic data for the genus *Brachinus*

Species and synonyms	2n	n	References
Brachinus (Brachinus) plagiatus Reiche	32	—	1, 7
B. (Brachinus) crepitans L.	39	18 + X	1, 7
B. (Brachinolomus) longicornis Fairm.	34	15 + IV	7
	32	—	7
B. (Brachinidius) bodemeyeri Apfelb.	25	—	1, 7
B. (Brachinidius) explodens Duft.	—	18 + X?	3
B. (Brachinidius) sclopeta F.	32	15 + XY	1, 7
	31	14 + XXY	7
B. (Brachinidius) variventris Schauf.	32	—	1
B. (Brachinoaptinus) baeticus Ramb.	33	—	7
B. (Brachinoaptinus) pecoudi Puel	—	8 + XY	4
B. (Brachinoaptinus) pateri Puel	22 (female)	10 + XX	2
	22	—	7
	20	9 + XY	7
B. (Brachinoaptinus) andalusiacus Rambur	32	—	2
B. (Cnecostolus) exhalans Rossi	21	10 + X	1
B. (Aploa) humeralis Ahr.	28	13 + XY	1
B. (Aploa) sexmaculatus Dej	28	13 + XY	6
B. (Neobrachinus) janthinipennis Dej.	18	8 + XY	5
B. (Neobrachinus) cordicollis Dej.	18	8 + XY	5

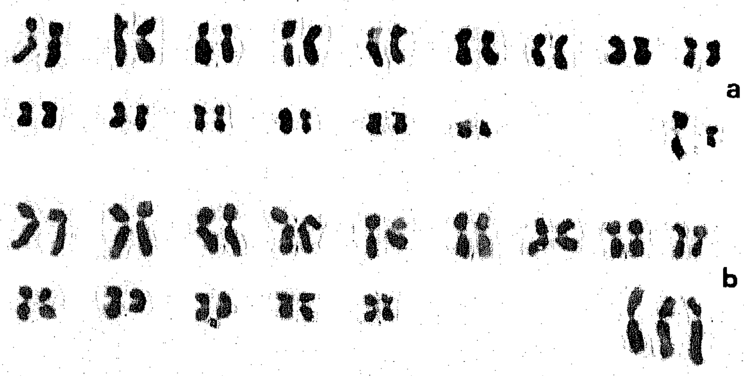
References: 1, Serrano (1981); 2, Serrano (1982); 3, Serrano (1986a); 4, Serrano (1986b); 5, Smith (1960); 6, Yadav *et al.* (1983); 7, present report.

The two males of *B. (Brachinidius) bodemeyeri* corroborate the diploid number 2n = 25, reported by Serrano (1981). The karyogram (Figure 6) is made up of meta- and submetacentric chromosomes, with an asymmetric chromosome which may be the X chromosome.

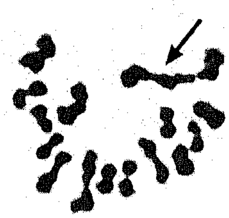
B. (Brachinidius) sclopeta shows a numerical polymorphism since of the eleven individuals studied eight of them have 2n = 31 and three have 2n = 32. The karyogram of the individuals with 2n = 32 (Figure 7a) is made up of meta- and submetacentric chromosomes in which the three largest pairs are of asymmetric morphology. The X chromosome is identified as a metacentric element about the size of the largest pair and the Y chromosome may be submetacentric and of the size of the ninth pair. The haploid number at metaphase I is n = 16. The karyogram of the individuals with 2n = 31 (Figure 7b) is in agreement with the others except for the lack of the Y chromosome. In addition the two largest chromosomes seem to have long arms of different sizes. Metaphase I cells have fourteen bivalents and a trivalent possibly made up of the largest pair and the X chromosome (Figures 8 and 9). Metaphase II cells are of two types with n = 15 and with n = 16.



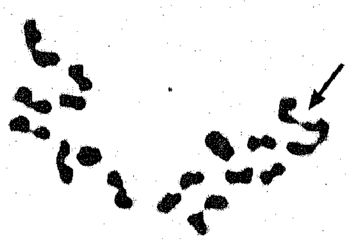
6



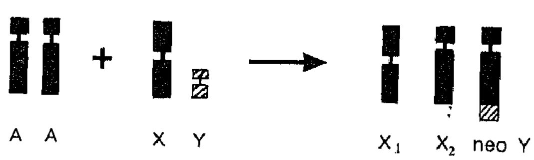
7



8



9

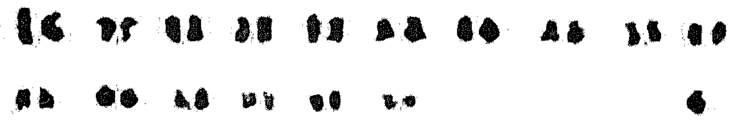


10

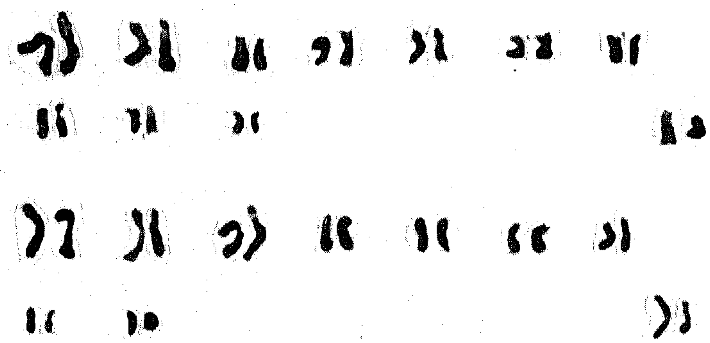
Figure 6 *Brachinus bodemeyeri*. Karyogram, 2n = 25. x2,000.

Figures 7 to **9** *B. sclopeta*. Karyograms with 2n = 32 (Figure 7a) and with 2n = 31 (Figure 7b) and metaphases I (Figures 8 and 9) with 2n = 31 showing the trivalent (arrow). x2,000.

Figure 10 Scheme showing a possible origin of the sex trivalent of *Brachinus sclopeta*.



11



a

b

12

13

Figure 11 *Brachinus baeticus*. Karyogram, 2n = 33. x2,000.

Figures 12 and **13** *B. pateri*. Karyograms with 2n = 22 (Figure 12a) and with 2n = 20 (Figure 12b); and metaphase I (Figure 13) with 2n = 20. x2,000.

The single male studied of *B.* (*Brachinoaptinus*) *baeticus* shows spermatogonial metaphases with 2n = 33 chromosomes. The tentative karyogram is made up of meta- and submetacentric chromosomes gradually decreasing in size (Figure 11).

The chromosome number of ten individuals of *B.* (*Brachinoaptinus*) *pateri*, is 2n = 20, whereas one individual has spermatogonial metaphases with 2n = 22. The difference between the two karyograms is in the third and eighth pairs of the individual with 2n = 22 (Figure 12a), and the large third pair of the individuals with 2n = 20 (Figure 12b). In metaphase I cells there are n = 10 bivalents (Figure 13). An heteromorphic pair observed in some cells may be the sex bivalent.

Discussion

The seven species studied possess a karyotype with a predominance of submetacentric chromosomes mainly among the largest pairs. Subtelocentric chromosomes are evident in *Brachinus crepitans*, *B. pateri* and *B. longicornis*. A large pair, generally submetacentric, with a marked difference in size from the following pair occurs in the first two species, *B. plagiatus* and *B. bodemeyeri*. Therefore, the karyotype of these species shows the characteristics found in most species of carabid beetles. The sex chromosome system of the species is XO or XY except for some individuals of *B. sclopeta*. In this species the trivalent observed in meiosis may be interpreted as the result of a fusion between the primitive Y with an autosome in individuals with 2n = 30 + XY chromosomes (Figure 10). This interpretation agrees with the observation that the three largest chromosomes are different in shape.

The mitotic and meiotic results of *B. longicornis* indicate that the species has a very high level of structural and numerical polymorphism. It has not been determined whether an heteromorphic bivalent present in two individuals corresponds to the typical XY pair of many *Brachinus* species. The chromosomes involved in the numerical polymorphism have not been determined. It is possible that they are not involved in the origin of the tetravalent, because some tentative observations suggest that this multivalent occurs both in individuals with 2n = 34 and 2n = 32. If it were confirmed as the general occurrence of the tetravalent then it may be a rare case of an XXYY system. Therefore, this species merits further investigation of several populations, because it shows the high degree of karyotypic dynamism which can be found in some carabid taxa such as *Melanius nigrita* (Serrano, 1981; Koch, 1985).

The numerical polymorphism of *B. pateri* [*B. obscuratus* Mateu; in Serrano (1982) and subsequent citations] can be explained as the result of a fusion between two pairs of autosomes, perhaps the third and eighth pairs of individuals with 2n = 22. This number, also reported by Serrano (1982) for females, indicates that the numerical polymorphism of the species may be geographically widespread.

The chromosome numbers found at present in the genus *Brachinus* (Table 2) support the hypothesis of Serrano (1982, 1986a) in which it is postulated that 2n = 32 may be the basic number of the genus, with a trend towards numerical decreases alternating the XO and neoXY systems through successive cycles of fusion autosome-heterosome and degeneration of heterosomes. The main role of the sex chromosomes in the karyotypic evolution of the genus is illustrated by the finding of a sex trivalent in *B. sclopeta*.

Recent results show, however, that there is an alternative trend in numerical changes towards higher numbers than 2n = 32. This is the case of the numerical polymorphism of *B. longicornis* (2n = 34), *B. baeticus* (2n = 33) and *B. crepitans* (2n = 39). The karyotype of the latter species includes at least five short-armed pairs, thus giving a clue to the origin of these numbers through dissociations or fissions. Both trends may be present in the same phyletic line at the subgenus level, as exemplified (Table 2) by *Brachinoaptinus*.

Acknowledgements

The authors wish to thank Mr J. L. Lencina, Mr F. Ochotorena and Mr J. de Ferrer for their help in collecting specimens. This research was supported by project number PB87-0584-CO2-02 of the Dirección General de Investigación Cientifica y Tecnica (DGICYT) of the Spanish Government.

References

KOCH D. 1985. Morphologische und öko-physiologische Differenzierung, Speziation und Verbreitung der Arten des *Pterostichus nigrita*-Komplexes (Coleoptera, Carabidae). Inag. Diss. Erlang. Doktorw. Math-Naturwiss. Fak, Universität zu Köln.

SERRANO J. 1981. Chromosome numbers and karyotypic evolution of Caraboidea. *Genetica* **55** 51–60.

SERRANO J. 1982. New chromosome number of Spanish Caraboidea (Coleoptera, Adephaga). *Genét. Ibér.* **34** 63–82.

SERRANO J. 1986a. A karyotypical approach to carabid evolution (Coleoptera). *In* Carabid Beetles, their Adaptations and Dynamics. pp 221–34. Edited by P. J. Den Boer, M. L. Luff, D. Mossakowski and F. Weber. Springer, Stuttgart.

SERRANO J. 1986b. A chromosome study of twenty species of Spanish carabids beetles (Coleoptera). *Genetica* **69** 133–42.

SMITH S. G. 1960. Chromosome numbers of Coleoptera. II. *Can. J. Genet. Cytol.* **2** 66–88.

WEBER F. 1968. Die intrapezifische Variabilität des heterochromatischen Armes eines Chromosomes beider Gattung *Carabus* L. (Coleoptera). *Chromosoma* **23** 288–308.

YADAV J. S., Kondal K. and Yadav A. S. 1983. Karyological investigations of Brachinini (Caraboidea: Coleoptera). *Nucleus* **26** 123–8.

Accepted 10 August 1990