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A cytogenetic study of the leaf beetle genus *Cyrtonus* (Coleoptera, Chrysomelidae)

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Abstract

The chromosomes of ten species of *Cyrtonus* and the genome sizes of six are surveyed. Among the total of 15 chromosomally studied species, 11 have $2n = 28$ chromosomes and a $13 + Xy_p$ male meioformula, three have $2n = 40$ and $19 + Xy_p$ and one $2n = 46$ and $22 + Xy_p$. All but one species with 28 chromosomes show only metacentric or submetacentric chromosomes, whereas the species with 40 and 46 chromosomes display some telocentrics or subtelocentrics, that are probably derived from the former by centric fissions. However, since the number of major chromosome arms is strikingly higher in these latter species (NF = 70 and 78) than in the 28-chromosome species (mostly NF = 56), other chromosomal rearrangements such as pericentric inversions or heterochromatin accretions could also be involved. The genome sizes display a narrow range, from 1C = 0.6–1.22 pg, and they are not significantly correlated with the chromosome numbers. Some possible factors implied in the rough chromosomal evolution of *Cyrtonus* are discussed in relation to a few other genera of the subfamily Chrysomelinae.

Introduction

The *Cyrtonus* leaf beetles (Coleoptera, Chrysomelidae) are a group of some 40 closely related species (Winkler, 1930; Cobos, 1953, 1954), distributed mainly in the mountains of the Iberian Peninsula. Three of the species reach the south of France and two are endemics, one each in Morocco and the Balearic Islands.

The apterous character of these rare beetles greatly reduces their capacity for dispersal. It is quite common that these species show restricted areas of distribution in the neighbour mountain ranges of almost all great Iberian reliefs and even in the highly xeric areas of southeastern Spain. The *Cyrtonus*, like most of the genera of the subfamily Chrysomelinae, are trophic specialists feeding as adults and larvae on the leaves of various Asteraceae plants (*Artemisia, Dittrichia, Helichrysum, Hieracium, Hyoseris, Lappa, Leontodon* and *Santolina*). The taxonomy of many of currently described species is misleading due to similar morphological features, uniform chromatic pattern of mostly dark green or bronzeous colours, and even the probable description of the two sexes under different names in some species (Cobos, 1954). Moreover, there is not a phylogenetic hypothesis to illustrate their presumed interrelationships. Thus the present analysis is aimed at the cytogenetic characteristics based on an examination of their karyotypes and DNA-content of their Feulgen-stained spermatids.

Previous findings have reported the chromosomal complements of five species (Petitpierre, 1978, 1984; Petitpierre & Segarra, 1985; Petitpierre, Segarra & Juan, 1993), four of them showing $2n = 28$ and one with $2n = 40$ chromosomes, and the haploid DNA content of only one of these species (Petitpierre, Segarra & Juan, 1993). We add herein the results on ten further species and new data on three previously examined. We discuss their chromosomal resemblances, the possible main evolutionary changes producing these karyotypes and those implied in the shifts of genome size.

Table 1. Cytogenetically analysed species of *Cyrtonus* and their sources

C. arcasi Fairmaire	Sierra de Guillimona, 1700 m alt. (Granada, SP)
Cyrtonus sp.	Sierra de Almijara: Puerto del Collado, 980 m alt. (Granada, SP)
C. contractus Fairmaire	Sierra Nevada: Trevenque basis, 1800 m alt. (Granada, SP)
C. cupreovirens Pérez	Moncayo submit: 2250–2300 m alt. (Zaragoza, SP)
C. cylindricus Marseul	Puerto del Pinar, 1900 m alt. (Granada, SP)
	Sierra de Guillimona, 1700 m alt. (Granada, SP)
C. elegans Germar	Cabo de Sâo Vicente (Algarve, PO)
C. fairmairei Rosenhauer	Sierra de las Nieves: Puerto de los Pilones, 1700 m alt. (Málaga, SP)
C. majoricensis Breit	Teix, 950 m alt. (Majorca, Balearic Islands, SP)
C. pardoi Cobos	Sierra de Villafuerte, 1800 m alt. (Albacete, SP)
C. plumbeus Fairmaire	Nacimiento (Almería, SP)
C. puncticeps Fairmaire	Frías de Albarracín, Km. 8.5 W, 1100 m alt. (Teruel, SP)
C. rotundatus Herrich-Schäffer	Ile Ratonneau (Bouches-du-Rhône, FR)
C. ruficornis Fairmaire	Puerto de Oncala, 1500 m alt. (Soria, SP)

SP = Spain, PO = Portugal, FR = France.

Materials and methods

The 13 species examined here and their geographic sources are given in Table 1. Adult male specimens were killed with acetic ether or ethyl acetate, their testes dissected on slides, teased with insect needles and then squashed under coverslips after 10–15 min in 45% acetic acid. The slides were frozen by immersion into liquid nitrogen, and the coverslips were later removed by a fine scapel. Finally, the dried slides were stained in 2–4% Giemsa in tap water for 15–20 min. Some unstained slides obtained by the same method were kept apart for measuring the DNA content of Feulgen-stained spermatids. The technique used for these analyses was that reported by Juan and Petitpierre (1989), and the flour beetle *Tribolium castaneum* (1C = 0.208 pg) was taken as an internal standard in each experiment. The light absorbance and the spermatid areas of the four examined species of *Cyrtonus* were measured by an image analyser VIDAS-21, coupled to a ZEISS AXIOSKOP microscope. One to seven individuals per species were examined in our chromosomal studies and one to three individuals for those of genome size.

Results

Among the ten presently examined species of *Cyrtonus*, seven displayed $13 + Xy_p$ male meioformulas and $2n = 28$ chromosomes, two showed $19 + Xy_p$ and $2n = 40$, and one $22 + Xy_p$ and $2n = 46$ chromosomes. The species with a diploid number of $2n = 28$ usually have karyotypes defined by metacentric chromosomes without telocentrics or subtelocentrics, such as those of *C. ruficornis* (Figure 1(a and b)), *C. cupreovirens* (Figure 1(c and d)), *C. cylindricus* (Figure 1(h)), *C. cobosi* (Figure 2(a)) and *C. elegans* (Figure 2(b)). Hence, all species studied thus far with $2n = 28$ chromosomes display metacentric elements and a fundamental number (FN, number of major chromosome arms) of FN = 56 out of *C. contractus* (see legends Figure 2(c)) which has five telocentric/subtelocentric chromosome pairs and a lower FN = 48 (Tables 2 and 3). Furthermore, the meiotic metaphases I obtained in some of these 28-chromosome species, namely those of *C. elegans* (Figure 1(e)) and *C. rotundatus* (Figure 1(g)), are in agreement with the previous findings.

The relative lengths and chromosome shapes of the karyotypes of *Cyrtonus* sp., *C. elegans* and *C. contractus* are reported in Table 3 and Figure 2. These chromosome sets are slightly asymmetric. The size of the largest autosome pair is three- to four-fold that of the smallest one. The y-chromosomes are dot-like elements that are even smaller than the lesser autosome pairs. The karyotypes of *C. cobosi* and *C. elegans* have very similar chromosomes, displaying mostly metacentrics with a few submetacentrics including the X-chromosome of each one. However, the karyotype of *C. contractus* differs clearly from the previous species as mentioned above and shown in Table 3.

C. arcasi, C. puncticeps and *C. fairmairei* share a $19 + Xy_p$ male meioformula and $2n = 40$ chromosomes. The karyotype of *C. arcasi* is illustrated at meiotic metaphase I (Figure 1(f)). That of *C. puncticeps*

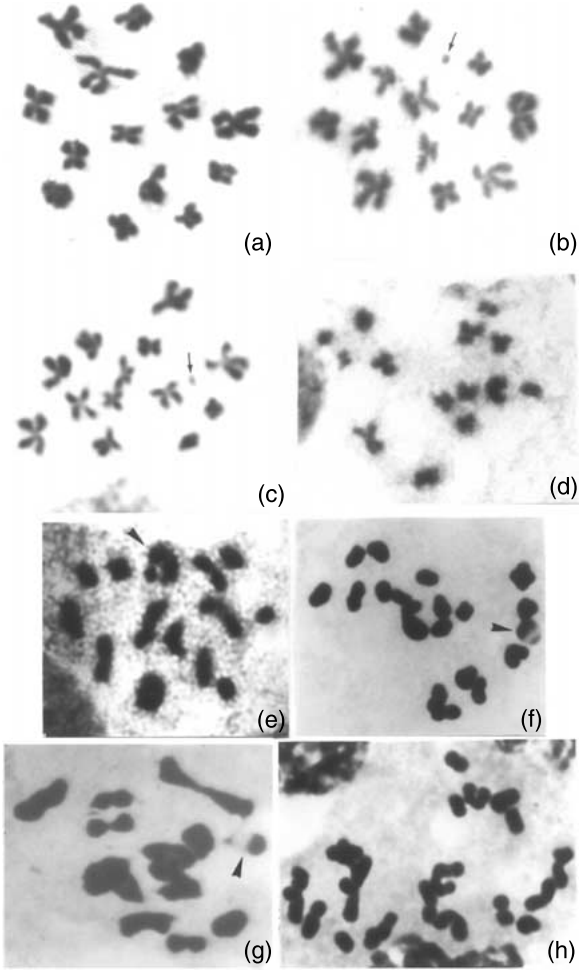

Figure 1. Meiotic metaphases II of *C. ruficornis* X-class (a) and y-class (b), *C. cupreovirens* y-class (c) and *C. elegans* X-class (d), all with $n = 14$ metacentric chromosomes. Meiotic metaphases I of *C. elegans* with $13 + Xy_p$ (e), *C. arcasi* with $19 + Xy_p$ (f), and *C. rotundatus* with $13 + Xy_p$ (g) meioformulas. Mitotic metaphase of *C. cylindricus* with $2n = 28$ chromosomes (h). The y-chromosomes are arrowed and the Xy_p are pointed by arroweads. All micrographs at 2000×.

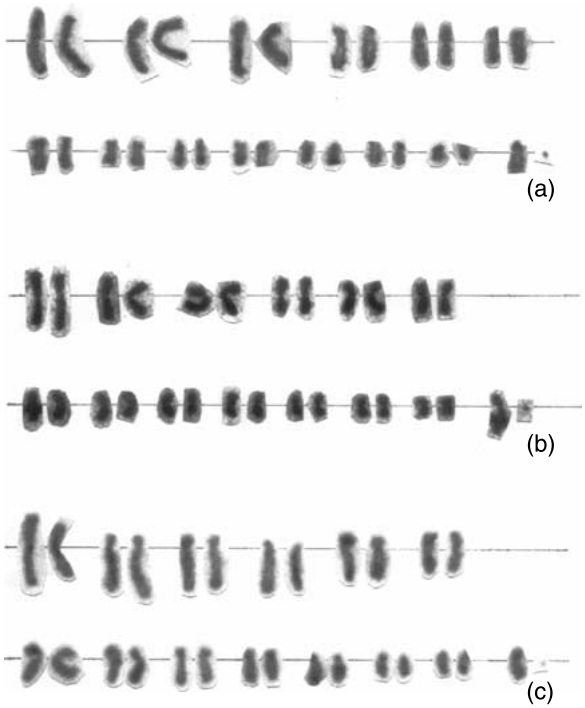

Figure 2. Karyotypes of *Cyrtonus* sp. (a) and *C. elegans* (b) with 28 chromosomes, all of them metacentrics or submetacentrics, and of *C. contractus* (c) also with 28 chromosomes but with five telocentric/subtelocentric autosome pairs. The X and y sex-chromosomes are at the lower right of each karyotype. All karyotypes at 2000×.

has been studied at metaphases I (Figure 3(a)), metaphases II (Figure 3(b)) and mitotic metaphases (Figure 3(c)). It is composed of five subtelocentric pairs which stand out among the remaining metacentric or submetacentric chromosomes. One medium sized pair of this latter group is conspicuously satellited (Figure 3(c)). The chromosomes from *C. fairmairei* have been examined at metaphases I (Figure 3(d)) and metaphases II (not shown). The karyotype is comprised of five subtelocentric pairs among the remaining metacentric or submetacentric pairs as occurs in *C. puncticeps*. Thus both species are characterized by a fundamental number of FN = 70 (Table 2).

The species displaying the highest diploid number is *C. pardoi* with a $22 + Xy_p$ meioformula (Figure 3(e and f)), that is $2n = 46$ chromosomes. Its karyotype (not shown) is composed of seven telocentric or subtelocentric chromosome pairs and 16 metacentrics or submetacentrics. Thereby the fundamental number of FN = 78 is the highest for the genus *Cyrtonus* (Table 2).

Seven meiograms from species of *Cyrtonus* are represented in Figure 4, where the metaphase I bivalents are arranged by their sizes: three of them having $13 + Xy_p$, another three with $19 + Xy_p$, and one with $22 + Xy_p$ meiformula. All display the 'parachute-like' sex-chromosome system (Xy_p). Most autosomal bivalents are unichiasmate rods whereas the bichiasmate ring-bivalents are very scarce (see Figures 3(a) and 4(b)). Three species with high chromosome numbers, *C. arcasi*, $2n = 40$ (Figure 4(d)), *C. fairmairei*, $2n = 40$ (Figure 4(e)) and *C. pardoi*, $2n = 46$ (Figure 4(g)), have bivalents of sizes smaller than

Table 2. Chromosomal and genome size data of *Cyrtonus*

	Chromosomal data				Genome size data	
	Male meioformula	2*n*	FN	No. ind.	1C (pg)	No. ind./cells
C. arcasi Fairm.	$19 + Xy_p$ [a]	40	–	2	1.22 ± 0.02	1/40
Cyrtonus sp.	$13 + Xy_p$	28	56	2	–	–
C. contractus Fairm.	–	28	48	1	–	–
C. cupreovirens Pérez	$13 + Xy_p$	28	56	2	1.17 ± 0.02	1/35
C. cylindricus Mars.	$13 + Xy_p$	28	56	5	–	–
C. elegans Germ.	$13 + Xy_p$	28	56	6	–	–
C. fairmairei Rosenh.	$19 + Xy_p$	40	70	2	0.91 ± 0.02	1/40
C. majoricensis Breit	$13 + Xy_p$ [b]	28	56	2	0.60 ± 0.01	3/75
C. pardoi Cobos	$22 + Xy_p$	46	78	7	1.21 ± 0.05	1/50
C. plumbeus Fairm.	$13 + Xy_p$ [b]	28	56	2	1.18 ± 0.04	1/22
C. puncticeps Fairm.	$19 + Xy_p$	40	70	2	–	–
C. rotundatus Herr. Sch.	$13 + Xy_p$	28	–	1	–	–
C. ruficornis Fairm.	$13 + Xy_p$	28	56	2	0.90 ± 0.02	2/40

FN = fundamental number (number of major chromosome arms).
[a] Petitpierre, Segarra and Juan (1993).
[b] Petitpierre et al. (1988).

Table 3. Relative lengths and shapes of chromosomes in three species of *Cyrtonus*

Chromosome	*Cyrtonus* sp.	*C. elegans*	*C. contractus*
1	13.8/m	11.9/m	11.2/m
2	12.7/m	9.9/m	10.6/st
3	11.5/sm	8.8/m	10.0/st
4	8.6/m	8.1/m	9.1/t
5	7.3/m	7.4/m	7.9/sm
6	6.5/m	7.2/m	7.4/m
7	5.3/m	6.7/m	7.3/sm
8	5.2/m	6.5/m	7.0/sm
9	4.9/m	5.9/m	6.5/sm
10	4.5/m	5.3/m	5.4/sm
11	4.4/m	4.8/m	4.6/st
12	3.7/m	4.5/m	3.5/st
13	3.2/m	3.8/m	3.4/m
X	6.5/sm	8.7/sm	5.4/m
Y	1.6/m	1.2/m	0.7/m

m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric.

those found in the species with $13 + Xy_p$, $2n = 28$ chromosomes (Figure 4(a–c)).

Finally, the values of genome sizes obtained from the Feulgen-stained spermatids in seven species of *Cyrtonus* show a moderate two-fold range, from 1C = 0.60 pg. in *C. majoricensis* to 1C = 1.22 pg. in *C. pardoi* (Table 2). Genome size does not seem to be significantly correlated with the chromosome

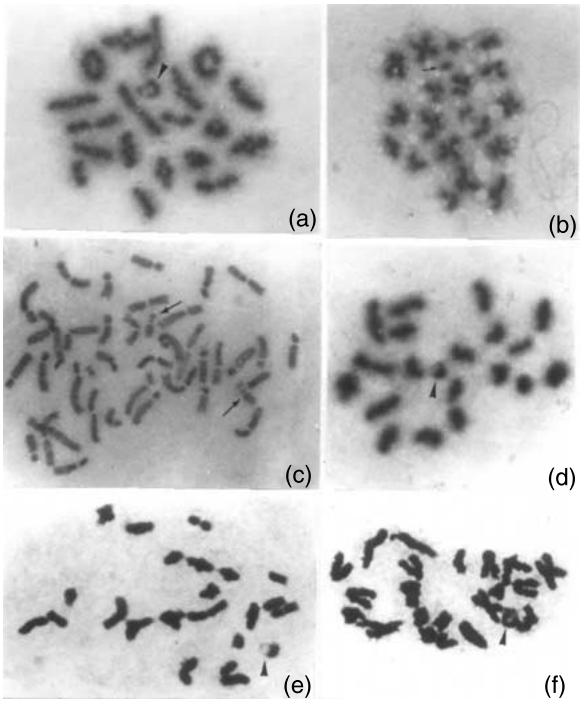

Figure 3. Meiotic metaphase I of *C. puncticeps* with $19 + Xy_p$ (a), metaphase II y-class (b) and spermatogonial metaphase (c) of *C. puncticeps* with 20 and 40 chromosomes, respectively. In (c), the pair of satellited medium-sized chromosomes are arrowed. Metaphase I of *C. fairmairei* with $19 + Xy_p$ (d) and *C. pardoi* with $22 + Xy_p$ (e), and diakinesis of *C. pardoi* also with $22 + Xy_p$ (f). The Xy_p are pointed by arrowheads, the y-chromosome by a small arrow and the satellited autosome pair of *C. puncticeps* by large arrows. All micrographs at 2000×.

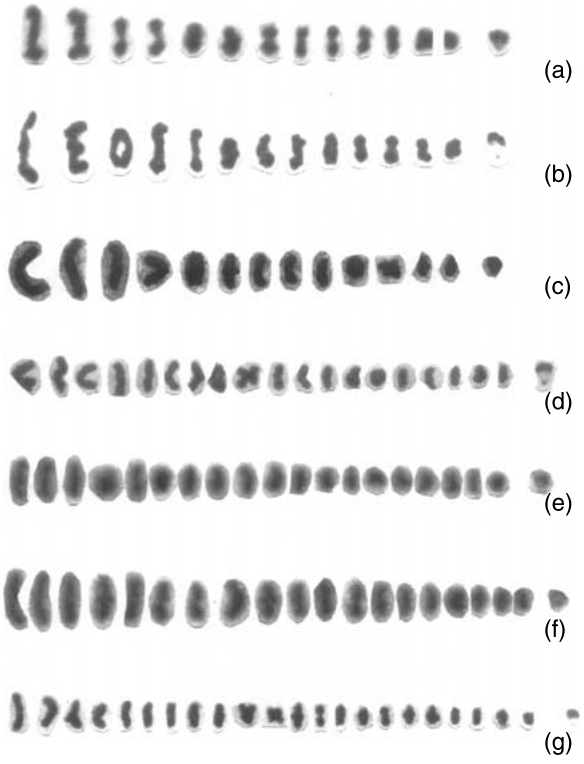

Figure 4. Meiograms of *C. cupreovirens* (a), *C. ruficornis* (b) and *C. elegans* (c) with $13 + Xy_p$, *C. arcasi* (d), *C. fairmairei* (e) and *C. puncticeps* (f) with $19 + Xy_p$, and *C. pardoi* (g) with $22 + Xy_p$. Note that almost all bivalents are unichiasmate. The Xy_p sex-chromosome bivalents are at the right of each meiogram. All micrographs at 2000×.

numbers. However, no species with a high chromosome number has a low value of genome size.

Discussion

With the present chromosomal results, a total of 15 species or 35% of described *Cyrtonus* have been analysed. Cobos (1954) recognized two morphological groups of species within *Cyrtonus*, those with 'elongated' outlines and those with 'round shortened' outlines. Because the modal chromosome number, $2n = 28$, is shared by taxa in both groups it could tentatively be considered as ancestral for the genus.

The modal and presumably ancestral chromosome number for *Cyrtonus* of $2n = 28$ is not shared by other cytogenetically well-known genera of the Chrysomelinae subfamily, such as *Timarcha* with a modal number of $2n = 20$, *Chrysolina, Oreina, Calligrapha* and *Gonioctena* with $2n = 24$ or $2n = 23$ in males, *Leptinotarsa* with $2n = 35$ in males, and *Chrysomela, Phratora* and *Phaedon* with $2n = 34$ (Petitpierre et al., 1988; Petitpierre, unpublished). Based only on these modal chromosome numbers, *Cyrtonus* is more closely related to *Chrysolina* and maybe other genera with diploid number 24 than to *Timarcha* and those genera with higher modal chromosome numbers. The results of our molecular phylogenies support this assumption regarding the interrelationship between *Cyrtonus* and *Chrysolina* (Garnería, Juan & Petitpierre, unpublished).

Bengtsson (1980) first used standard deviations (SD) of diploid chromosome numbers to measure chromosomal divergences as well as to discriminate between genera of high versus low rates of chromosomal evolution. The *Cyrtonus* can be included among the beetle genera having a high rate of chromosomal evolution, because the SD of the 15 checked species is 6.33, in agreement with similar high values found in other related leaf beetle genera, such as *Timarcha* and *Chrysolina* (Petitpierre, Segarra & Juan, 1993). These three genera are composed of apterous (*Cyrtonus* and *Timarcha*) or winged but non-flying species (most *Chrysolina*), a fact that might have some effect on the rate of chromosomal evolution. Conversely, other genera composed of flying species, typically have low or even null values of SDs (Petitpierre, Segarra & Juan, 1993).

The low vagility of *Cyrtonus* is likely partially responsible for this high rate of chromosomal evolution, if we take into account the fixing effects of genetic drift and/or inbreeding on the newly arisen non-deletereous chromosomal mutations (Lande, 1979, 1985; Hedrick & Levin, 1984; Chesser & Baker, 1986). Although all currently studied species of *Cyrtonus* feed only on Asteraceae plants while the trophic range of host-plants in *Timarcha* and *Chrysolina* is much more heterogeneous, on six and seven botanic families respectively, this difference has not constrained the rate of rough chromosomal evolution in *Cyrtonus* with respect to those of the two other genera. Even though the number of host-plant families was significantly correlated with the rates of chromosomal evolution per genera in Chrysomelinae leaf beetles (Petitpierre, Segarra & Juan, 1993), it seems clear that low deme sizes can be influenced either by a reduced vagility or by a specialized trophism, among other concurrent factors.

All the species with 13+Xy_p and 28 chromosomes, except *C. contractus*, show similar karyotypes consisting largely of metacentric chromosomes, as happens in *Cyrtonus* sp., *C. cupreovirens, C. cylindricus, C. elegans, C. ruficornis* and in that of *C. plumbeus* reported by Petitpierre and Segarra (1985). *C. contractus* differs from the previous species by having five pairs of telocentric/subtelocentric chromosomes and a fundamental number of NF = 48, instead of NF = 56 as shared by the remaining species with 28 chromosomes.

The species with higher chromosome numbers, *C. arcasi, C. fairmairei* and *C. puncticeps* with $2n = 40$, and *C. pardoi* with $2n = 46$, have likely been derived from the 28 chromosome species by six and nine presumed centric fissions, respectively. The five telocentric/subtelocentric chromosome pairs displayed by the karyotypes of *C. fairmairei* and *C. puncticeps*, and the seven of *C. pardoi* may be taken as proofs of these fission events. Nevertheless, since the fundamental number of the first two species, FN = 70, and that of the third, FN = 78, are clearly higher than the NF = 56 of most 28 chromosomes species, a fair number of the original telocentric/subtelocentric chromosomes, likely experienced subsequent pericentric inversions or heterochromatin accretions. This resulted in a shift to metacentrics/submetacentrics, thereby increasing their FNs. The karyotype of *C. puncticeps* is remarkable due to the presence of a satellited medium size autosome pair which likely holds the nucleolus organizing region (NOR), as it is usually found in animal chromosomes (Macgregor, 1993; Sumner, 2003). Nevertheless, since this type of satellite chromosome has only been detected in *C. puncticeps*, the NOR or NORs should be located in other kinds of chromosomes in the remaining species.

The 'parachute-like' Xy_p male sex-chromosome system is found in all the species of *Cyrtonus* where meiotic metaphases I have been observed. This system is the most common in the entire leaf beetle family as well as specifically in the subfamily Chrysomelinae (Petitpierre & Segarra, 1985; Petitpierre et al., 1988). Contrary to the constancy of Xy_p found in the male meiosis of *Cyrtonus*, other extensively analysed genera of Chrysomelinae display alternate sex-chromosome systems. For example, *Calligrapha* nearly always share the XO sex-chromosome system (Robertson, 1966; Vaio & Postiglioni, 1974), and *Leptinotarsa* has the XO as the unique sex-chromosome system (Hsiao & Hsiao, 1983). Moreover, although the XO system occurs in some species of *Chrysolina*, the great majority of them show the common Xy_p (Petitpierre et al., 1988; Petitpierre, 1999). This Xy_p sex-chromosome system is usually considered ancestral in Coleoptera except for those beetles in the suborder Adephaga (Smith, 1951, Smith & Virkki, 1978; Virkki, 1984). Thus, the XO and neo-XY sex-chromosome systems, also found in Chrysomelinae, presumably derived from the Xy_p by the loss of the y-chromosome giving rise to the XO, and from this latter system to the neo-XY by an autosome/X-chromosome reciprocal translocation (Blackman, 1995).

The few data obtained on the genome size in *Cyrtonus* are not consistent with taxonomic and evolutionary standpoints. The range of variation for the seven analysed species is narrow, from 1C = 0.60 to 1C = 1.22 pg, in comparison with another genus of Chrysomelinae, *Chrysolina* which shows a six-fold variation. However, variation in *Timarcha* is clearly much lower than in *Cyrtonus* (Petitpierre, Segarra & Juan, 1993). In addition, the two morphological groups of *Cyrtonus*, those of 'round shortened' and those of 'elongated' outline (Cobos, 1954), are not associated with separated values of genome size, rather these values overlap. Furthermore, the correlation between the genome size and diploid chromosome number, $r = 0.38$, is positive but statistically insignificant, even though all species with high chromosome number display high values of genome size. A previous study with a much larger sample of nearly 40 leaf beetles, belonging to a five distinct subfamilies (Petitpierre, Segarra & Juan, 1993), gave a similar coefficient of correlation, $r = 0.39$, although in this case the value was statistically significant ($P < 0.005$).

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