Genome size, chromosomes, and egg-chorion ultrastructure in the evolution of Chrysomelinae

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1. Introduction

The leaf beetles of the subfamily Chrysomelinae are mostly insects of medium size (5–20 mm. long), frequently shiny and often metallic, variable in shape, but with round and convex outlines prevailing. They are worldwide in distribution being especially abundant in temperate areas such as the Holarctic region and Australia, and comprise approximately 3000 species in 176 genera (Daccordi, 1983). The current taxonomy assumes two tribes for Chrysomelinae, Timarchini and Chrysomelini, the former with only one subtribe and the latter with twelve (Seeno & Wilcox, 1982; Daccordi, 1983).

The chromosomes are known for nearly 190 chrysomelines (Petitpierre *et al.*, 1988; Petitpierre, unpubl.), or 6.5% of those described, making them relatively the best surveyed subfamily of leaf beetles. Their main karyological evolution has presumably taken place by centric fissions and pericentric inversions to account for the frequent increases in chromosome number and the general occurrence of meta- or submetacentric chromosomes (Virkki, 1964; Petitpierre & Segarra, 1985; Petitpierre, 1988a). The three main stages of karyological divergence of Chrysomelinae are represented by the three modal haploid values of 10 (Timarchina), 12 (most checked subtribes), and 17 (Chrysomelina and Phyllodectina) and they show a remarkable correspondence with the principal types of larval morphology (Paterson, 1931; Takizawa, 1976; Cox, 1982) and the defensive substances secreted by the adults (Pasteels *et al.*, 1988).

In the present paper we shall report and discuss some findings on another cytogenetic feature, the genome size or nuclear DNA content, together with new chromosomal data and several insights into the chorion ultrastructure. Our aim is to achieve a better understanding of the genetic and evolutionary interrelationships among chrysomelines in the light of present classification.

2. Genome size in Chrysomelinae: present experimental data

The genome size is a species-specific genetic character which was formely considered invariant but it is nowadays assumed to show some intraspecific variation in a significant number of animals (Sherwood & Patton, 1982; Gold & Amemiya, 1987; Black & Rai, 1988; Ragland & Gold, 1989; Alvarez-Fuster *et al.*, 1991) and plants (Price *et al.*, 1980; Bennett, 1985; Greilhuber & Speta, 1985; Laurie & Bennett, 1985; Rayburn *et al.*, 1985). On the other hand, the minimum value of genome size per phylum or class is closely related with their morphological complexity and phylogenetic advancement (Lewin, 1990), even though many of these higher taxa can show a striking variation, a fact which has been called C-value paradox (for C constant species-specific value of genome size). The whole issue of variation in genome size among closely related species is still a subject of controversy (Cavalier-Smith, 1985; John & Miklos, 1988) but some nucleotypic or nucleoskeletal effects, solely due to the physical mass of DNA, seem rather well proved (Bennett & Smith, 1976; Bennett *et al.*, 1982; Cavalier-Smith, 1978, 1985; Levin & Fundenburg, 1979; Olmo, 1983; MacLaren *et al.*, 1989).

Our results on the genome size in chrysomelids deal only with interspecific variation because the low number of checked specimens per species does not usually allow the detection of intraspecific variation. The DNA content of Feulgen-stained spermatids has been measured in 45 species of leaf-beetles (Petitpierre *et al.* in press) by using a technique described elsewhere (Juan & Petitpierre, 1991). Among these examined taxa were 21 species of Chrysomelinae which showed a wide range of 1C genome sizes from 0.20 to 3.69 pg of nuclear DNA content. The scored values of genome sizes in these species are listed in Table 1. As can be seen from this table and from the histogram (Fig. 1) most species of Chrysomelinae have nuclear DNA contents between 0.7 and 1.1 pg. The modal values include the genome sizes of *Timarcha* and several *Chrysolina*, however,

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Table 1. mean genome sizes and chromosome numbers in 21 species of Chrysomelinae

	Source	1C (pg)	2n (♂)
Timarcha balearica Gory	Mallorca, Spain	0.914	22
T. fallax Pérez	Granada, Spain	0.813	20
T. intermedia H. S.	Amería, Spain	0.823	20
T. marginicollis Rosh.	Granada, Spain	0.833	20
Chrysolina affinis (F.)	Barcelona, Spain	0.837	24
C. americana (L.)	Mallorca, Spain	0.561	24
C. aurichalcea (Mann.)			
2n (♂) = 31 chr. sibling sp.	C. Honshu, Japan	0.813	31
2n (♂) = 41 chr. sibling sp.	C. Honshu, Japan	0.792	41
C. banksi (F.)	Mallorca, Spain	1.169	23
C. carnifex (F.)	Barcelona, Spain	3.692	40
C. herbacea (Duftschmidt)	Girona, Spain	0.905	24
C. peregrina (H.S.)	Mallorca, Spain	1.072	46
C. pyrenaica (Dufour)	Lleida, Spain	0.626	40
C. viridana (Kuester)	Mallorca, Spain	0.618	24
Oreina cacaliae (Schrank)	Lleida, Spain	0.987	24
Leptinotarsa decemlineata (Say)	Mallorca, Spain	0.460	35
Cyrtonus arcasi Pérez	Granada, Spain	0.749	40
Gastrophysa polygoni (L.)	Barcelona, Spain	0.350	24
Phaedon cochleariae (F.)	Barcelona, Spain	0.523	34
Chrysomela populi L.	Barcelona, Spain	0.346	34
Prasocuris junci (Brahm)	Barcelona, Spain	0.203	34

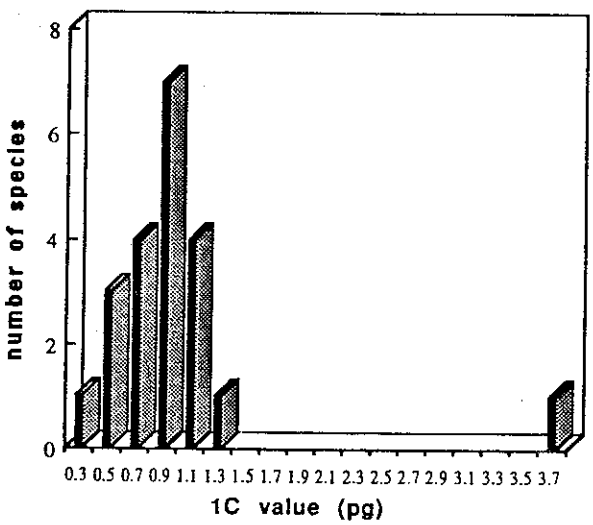


Fig. 1. Histogram of genome sizes in 21 species of Chrysomelinae leaf beetles.

the species of this latter genus exhibit a great range of 1C values from 0.56 to 3.69 pg. Closely related *Chrysolina* species with very similar karyotypes such as those of 2n(♂) = 23 or 2n = 24 chromosomes, which feed mainly on Lamiaceae, may differ clearly in their genome sizes, for example *C. banksi* and *C. americana* (1.17 and 0.56 pg) or *C. herbacea* and *C. viridana* (0.90 and 0.62 pg). So, the maintenance of similar chromosome sizes and shapes, from which it could be presumed a karyotypic stability, does not preclude drastic shifts in genome size as it has been reported in acridoid grasshoppers, where an apparently uniform set of acrocentric chromosomes conceals great differences in DNA amounts and of genome organization too (John & Hewitt, 1966; Hewitt, 1979).

Species which have recently diverged, such as the 31- and 41-chromosome siblings of the Japanese *Chrysolina aurichalcea*, agree in their spermatid DNA content of about 0.80 pg (Petitpierre *et al.*, 1991). Thus, the presumed unknown chromosomal arrangements involved in their origin have not changed the basic genome size of these siblings, in the same manner that they have kept a very high genetic similarity for allozymes, as reported by Sakanoue and Fujiyama (1987).

2.1 Evolutionary issues of genome size in insects and coleopterans

Hinegardner (1976) claimed that specialization within several higher taxa of animals, such as molluscs, holothurians, bony fishes, amphibians and mammals, was inversely correlated with genome size, being the specialization and low DNA content associated features of all these organisms. Although this trend can no longer be considered universal and some reverse cases of evolution by DNA increases have been well-illustrated (John & Miklos, 1988), it is a much more frequently encountered phenomenon than the opposite.

Insects are not exceptions to this common finding and Bier and Müller (1969) pointed out that ancestral insects namely Orthoptera display much larger genomes than the advanced namely Coleoptera and Diptera. Even though at present many more species have been scored the situation is the same as that of 25 years ago.

The mean 1C nuclear DNA content of 42 species of Orthoptera (9.78 pg) is strikingly higher than those

of 113 Coleoptera (0.63 pg), 10 Lepidoptera (0.94 pg) and 45 Diptera (0.85 pg). Some Coleoptera and Diptera hold some species having very small genomes for insects, with values lower than 0.2 pg, and among them that of the dipteran *Prodiamesa olivacea*, 0.13 pg, is the lowest so far recorded in insects (Fig. 2). Therefore, the holometabolous (endopterygote) insects have, in general, smaller genomes than hemimetabolous (exopterygote) insects, but all of them show a quite large span of values.

Within Coleoptera, the average genome sizes differ from family to family (Fig. 3). The Chrysomelidae (45 spp.) have a mean of 0.78 pg (Petitpierre *et al.* in press and unpubl.), the Tenebrionidae (52 spp.) 0.35 pg (Juan & Petitpierre, 1991; Alvarez-Fuster *et al.*, 1991), whereas Scarabaeoidea (Geotrupidae + Scarabaeidae) (7 spp.) 0.99 pg (Bosch, 1989), and Dermestidae (6 spp.) 1.39 pg (Fox, 1972). Scarabaeoidea and Dermestidae are more ancient taxa than Tenebrionidae and Chrysomelidae (Crowson, 1981), and this is in agreement with their higher mean amount of nuclear DNA though many more species of Scarabaeoidea should be analyzed before reaching a reliable conclusion.

2.2 Evolutionary issues of genome size in Chrysomelinae

There is a great range of variation in the genome size within subfamilies of Chrysomelidae. This was true for at least three species examined within the six subfamilies surveyed (Petitpierre *et al.* submitted). This range is extreme in Chrysomelinae (0.20–3.69 pg) but probably this is partly because of a larger sample in this subfamily than in the remainder. Has this variation any phylogenetic meaning?

The immature stages provide a valuable tool to trace the phylogenetic derivation of the Chrysomelinae. Their larvae are classified into three main groups: a) Timarchini, characterized by non-tuberculate larvae among other characters, b) Chrysolinina,

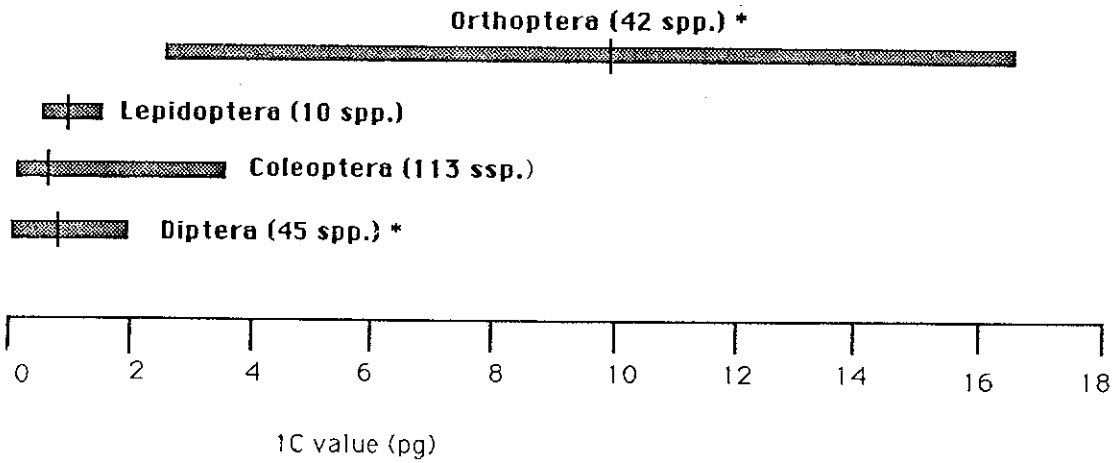


Fig. 2. Mean and range of genome sizes in four insect orders. Note the striking difference of the Orthoptera mean genome size with respect to the others. *Some species have heterogeneity of 1C nuclear DNA content. References: Belda *et al.* (1991), Bier & Muller (1969), Black & Rai (1988), Brown (1975, *in* Hewitt, 1979), Gosálvez *et al.* (1980), John & Hewitt (1966), Jost & Mameli (1972), Juan & Petitpierre (1991), Laird (1973), Manning *et al.* (1975), Rao & Rai (1987), Rasch *et al.* (1971), Rees *et al.* (1978), Samols & Swift (1979), Sparrow *et al.* (1972), Wilmore & Brown (1975), Zacharias (1979), Zacharias *et al.* (1982).

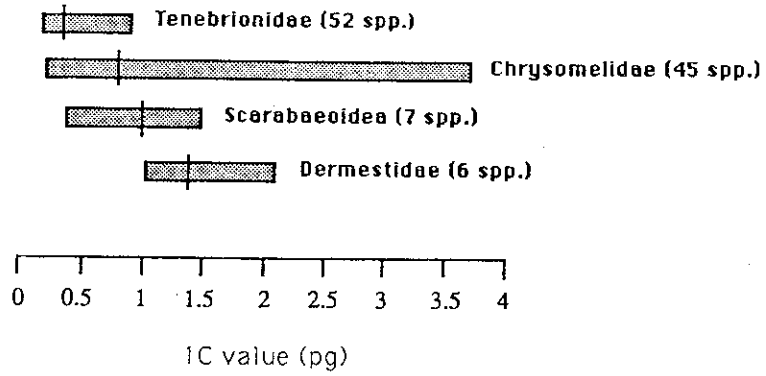


Fig. 3. Mean and range of genome sizes in four families or superfamilies of Coleoptera. References in the text.

Doryphorina and Gonioctenina, with tuberculate larvae devoid of glands, except for only one abdominal pair, and c) Chrysomelina (=Phaedonina) and Phyllodectina having also tuberculate larvae but here bearing multiple eversible glands able to secrete defensive substances (Paterson, 1931; Cox, 1982; Pasteels *et al.*, 1988). These three types of larvae correspond with the three modal chromosome numbers, 2n=20, 2n=24 and 2n=34, as pointed out in the introduction and elsewhere (Petitpierre & Segarra, 1985; Petitpierre, 1988a).

At first sight, the genome sizes of *Timarcha* (Timarchina) and *Chrysolina* (Chrysolinina) do not differ greatly since both genera share modal values about 0.8–0.9 pg. Nevertheless some species of *Chrysolina* deviate clearly from the mean and modal values, a fact which does not occur in *Timarcha*. Thus three species of *Chrysolina* (*C. americana, C. pyrenaica* and *C. viridana*) have 1C genome sizes lower than 0.65 pg, and one species (*C. carnifex*), has undergone a strong enlargement of its genome to 3.69 pg, the largest so far found in beetles. In *Chrysolina*, both decreases and increases in genome size are probably secondary events which can be traced from the presumed ancestral Chrysomelinae genome size of 0.8–0.9 pg. These events might be indicative of a more advanced evolutionary position of *Chrysolina* with respect to *Timarcha*, in agreement with adult and larval morphology, reproductive behaviour, and chromosome numbers.

Furthermore, when we compare the average genome size of 1.3 pg for Timarchina+Chrysolinina (15 species), it is significantly higher than that of five Chrysomelina (*Cyrtonus, Gastrophysa, Phaedon, Prasocuris* and *Chrysomela*) in which is 0.43 pg. Very likely the evolution from the former to the latter took place by a substantial decrease in genome size, even though one species of Chrysomelina, *Cyrtonus arcasi*, has a higher genome size than three species of Chrysolinina (*C. americana, C. pyrenaica* and *C. viridana*). This presumed decrease in the amount of the nuclear DNA in Chrysomelina parallels their 2n=34 modal chromosome number and the glanduliferous character of their larvae.

Leptinotarsa decemlineata, the Colorado potato beetle, is the only species of Doryphorina checked for genome size, it has a slightly larger genome (0.46 pg) than most Chrysomelina but smaller than all those examined in Timarchina and Chrysolinina. It could be claimed that Doryphorina were placed between Chrysolinina and Chrysomelina, but before assuming this intermediate position of Doryphorina at least some other species in this subtribe should be investigated to support this very preliminary assumption.

2.3 Nucleotypic correlates of genome size in Chrysomelinae

The simplest cytogenetic correlation that can be tested from data on genome size is that regarding the chromosome number. This kind of correlation can be expected in polyploid series, being quite common in the plant kingdom but rare among animals. However, in the absence of polyploidy there is no previous argument to assume such a correlation and examples of any type are well documented in animals (Hinegardner, 1976).

Our research on coleopterans has supplied positive and significant correlations between both cytogenetic characters in 44 species of Tenebrionidae (Juan & Petitpierre, 1991) and 39 species of Chrysomelidae (Petitpierre *et al.* submitted). The present much smaller sample of 21 species of Chrysomelinae shows a positive but not a significant interrelationship between both cytogenetic parameters, $r=0.203$, $0.2>P>0.1$. An increase in chromosome number does not necessarily correspond with an enlargement of genome size, what is mainly attributed to the reported rise of modal chromosome number of Chrysomelina species coupled with an average decrease of genome size. On the contrary, positive and highly significant coefficients of correlation between genome size and either spermatid area or total metaphase I chromosome area, were obtained in 39 checked species of Chrysomelidae (Petitpierre *et al.* in press).

Additional correlations in animals, other than those described between genome size and nucleus or chromosome area are much less grounded. Among them were the correlations found between genome size and adult body size in molluscs (Hinegardner, 1973), copepod crustaceans (McLaren *et al.* 1989), and *Dermestes* beetles (Fox, 1972), and those between genome size and hatch time in amphibians (Horner & Macgregor, 1983). However, the reliance of these latter correlations have been strongly criticized or even rejected as false by Larson (1984) and by John & Miklos (1988).

In the present concern of genome sizes of Chrysomelinae leaf beetles it should be pointed to that there is no correspondence between nuclear DNA content and body size. *Chrysolina carnifex* and *C. viridana* for instance, have similar, adult body lengths (6–9 mm) but the genome size of the former is more than six-fold that of the latter. In addition also, *C. banksi* and *Leptinotarsa decemlineata* have similar body lengths but the former has a genome size about twice that of the latter. Moreover, there is insufficient information to establish a possible relationship between genome size and time of development in

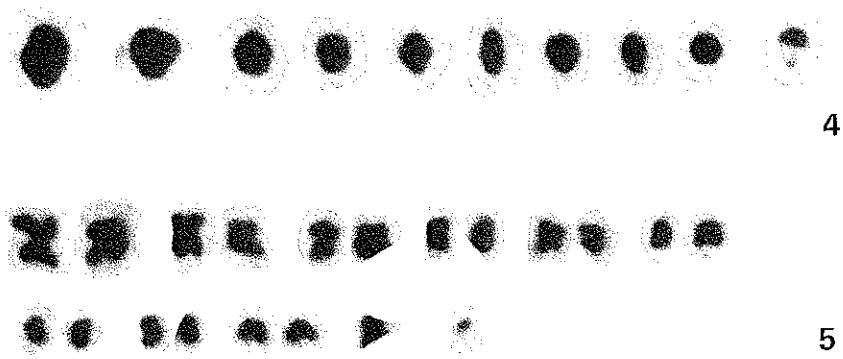
Chrysomelinae. Nevertheless, all species of *Timarcha* and *Chrysolina* possess genome sizes higher than 0.6 pg and most of them have only one generation per year whereas *Leptinotarsa decemlineata*, *Gastrophysa polygoni*, *Chrysomela populi* and *Prasocuris junci* whose genome sizes are lower than 0.5 pg have more than one generation per year in Southern Europe. Are the smaller genomes of this second group of species somehow associated with the existence of several generations per year? It seems probable, since the rate of cell metabolism as well as duration of DNA replication and cell division are inversely dependent on genome size (Szarski, 1976, 1983). The genome size plus the genotype probably have an immediate effect on the length of life cycle, that is on the speed of development. Whatever the direct interrelationships between genome size and duration of life cycle in chrysomelines could be, this feature is by no means universal for leaf beetles. However, small genomes also occur in the univoltine Clytrinae and Cryptocephalinae species examined (Petitpierre *et al.* in press). Besides that the genotypic contributions to these physiological traits, those dependent on the type and number of controlling genes, cannot be neglected and should be taken into account in this context.

3. New chromosomal findings on chrysomelines

A few species of chrysomelines have recently become available for chromosomal analysis and should be added to the list of Petitpierre *et al.* (1988), including some 180 taxa. The new data come from three species of *Oreina*, four of *Chrysolina*, and one of *Cyrtonus*. Together they providing further support of the presumed karyological divergence of Chrysomelinae which is stated above and that of *Chrysolina* in particular (Petitpierre, 1981, 1988a; Petitpierre & Segarra, 1985).

The most interesting finding of the present study is that of *Oreina viridis* (Duft.) where an individual from the Massif des Vosges (Haut-Rhin, France) showed a meioformula and karyotype of 9+Xyp, 2n=20 chromosomes. Such values are known only in several taxa of the mostly Mediterranean *Timarcha* (Petitpierre, 1970, 1976) and in a South American *Cosmogramma* species (Vidal, 1984). The chromosomes of *O. viridis* somewhat resemble those of taxa in the *Timarcha goettingensis* complex (Petitpierre, 1976). This is the presumed ancestre of the genus, because its 20 chromosome complement displays an asymmetry of sizes with two large pairs clearly distinguishable from one medium pair and the remaining small chromosome pairs. All of these chromosomes are metacentric except one small acrocentric pair. At metaphase I the sex-chromosomes of *O. viridis* form a typical parachute shape, Xyp, and the y-chromosome is the smallest element of the set (Figs. 4 and 5). The karyotypes of taxa in *Timarcha goettingensis* complex differ from that of *O. viridis* in having three, instead of two large autosomes and a large X-chromosome, as the most obvious differences.

Two other species of *Oreina*, *O. speciosa* (L.) (=*O. gloriosa* auct.) from the Massif des Vosges (Haut-Rhin, France) and *O. speciossisima* (Scop.) from Val d'Aran (Pyrenees of Lleida, Spain), display the modal meioformula and karyotype (11+Xyp, 2n=24 chromosomes) found in almost all *Oreina* and in many species of the closely allied genus *Chrysolina*. This meioformula, was also observed in a specimen of *Chrysolina graminis* (L.) from Bidache (Pyrenées Atlantiques, France), showing medium and small meiotic bivalents at metaphase I and some acrocentric chromosomes at spermatogonial metaphases. This contrasts with the common rule of metacentry-submetacentry found in closely related *Chrysolina* species feeding on Lamiaceae (Petitpierre, 1983). In addition, *Chrysolina gypsophilae ripoceanensis* Bourdonné, from Bains d'Uchet (Landes, France) showed a meio-



Figs. 4–5. Chromosomes of *Oreina viridis*: Meiogram of Metaphase I bivalents, the Xy_p is at the extreme right (4) and karyogram of spermatogonial metaphase chromosomes, the y-chromosome is at the right lower row (5).

formula and karyotype respectively of 10+Xyp and 2n=22 chromosomes, which do not differ with respect to those of *C. kuesteri* (Hell.) from either Catalonia (Petitpierre, 1981, 1983) or S.W. France (Petitpierre, unpubl.) However, since the karyotype of *C. gypsophilae grossepunctata* (Har. Lind.) from Tenerife (Canary Is.) has 2n=30 chromosomes and a 14+Xyp meioformula (Petitpierre, 1982; Petitpierre *et al.*, 1988), it is evident that the latter cannot be conspecific with *gypsophilae ripoceanensis* and should be better renamed as *C. grossepunctata* or as *C. lucidicollis grossepunctata*, the latter following a suggestion by Bourdonné and Doguet (1991).

One specimen of *C. (Allochrysolina) fuliginosa galii* (Weise), from Lesparrau (Ariège, France) showed a 20+Xyp meioformula made of similar size bivalents. This species is allied by external morphology and choice of food-plants *C. analis* (L.), *C. carnifex* (F.), *C. curvilinea* (Weise) (=*C. janbechynei* Cobos) and *C. marginata* (L.), included in the subgenus *Chalcoidea* (Bourdonné & Doguet, 1991). The chromosome number 2n=42, of *C. fuliginosa*, reinforces its close interrelationship with the above species which all have the quite similar 2n=40 chromosomes and 19+Xyp meioformula.

The genus *Cyrtonus*, an almost endemic Iberian taxon in the subtribe Chrysomelina, is karyologically known for four species all sharing 28 chromosomes and a 13+Xyp meioformula (Petitpierre *et al.*, 1988). Taking into account this number and 2n=34 being the modal value for the subtribe Chrysomelina, we suggested that *Cyrtonus* should be placed in an intermediate position within the presumed evolutionary branching of the subtribe (Petitpierre, 1988a). We have presently analyzed a fifth species, *C. arcasi* Pérez, from La Sagra (Granada, Spain), which has 40 chromosomes and a 19+Xyp meioformula. This species deviates strikingly from other *Cyrtonus* spp. in chromosome number although there is no morphological correspondence with this karyological feature as would be expected. Since all *Cyrtonus* are apterous species with a noteworthy rate of endemism in the Iberian Peninsula (about 40 species), it is not surprising to find a karyotype clearly distinct from the others, whose origin can be probably ascribed to the fixation of chromosomal mutations in small demes by effect of inbreeding and genetic drift.

To summarise, the new chromosomal data on Chrysomelinae leaf beetles do not fundamentally alter the main picture of their karyological derivation, except that the ancestral beetle meioformula of 9+Xyp (2n=20 chromosomes) is no longer unique to *Timarcha* since it has now been found in other species, such as *Oreina viridis* (Chrysolinina), and as reported by Vidal (1984) in *Cosmogramma decora* Stal. (Doryphorina). These findings further support the ancestral stage of 9+Xyp (2n=20) for Chrysomelinae, despite the prevailing occurrence of 11+Xyp (2n=24), being the 9+Xyp so far encountered in species of three different subtribes, which are not considered as very apomorphous on morphological grounds.

4. Egg-chorion ultrastructure

The eggs of insects, like those of some other animals, have a more or less hardened shell which protects them and the developing embryos from possible detrimental external effects, either physical or biological. This eggshell is made of certain kind of maternal proteins, the chorion proteins, encoded by a family of genes whose products assemble and crosslink to form a multilayered complex known as the egg-chorion or simply chorion (Watson *et al.*, 1987). The outside appearance of the egg-chorion can be properly revealed by scanning electron microscopy (SEM) which has provided an amazing array of relief ultrastructures often used as taxonomic characters (Hinton, 1981). Although the causative relationships between egg-chorion proteins and these ultrastructures have not been generally elucidated out, it is clear that they are directly dependent on the aminoacid sequence of these proteins, which indeed derives from the gene information, and therefore, these ultrastructures can be used to ascertain genetic homologies among allied species.

The eggs of beetles are commonly constituted of a rather smooth and soft surface contrary to those of most Lepidoptera and Hemiptera which are much more hardened and sculptured (Crowson, 1981). Very few papers have been published on the egg-chorion ultrastructure of beetles (Klausnitzer & Forster, 1971; Mazzini, 1974, 1975; Biemont *et al.*, 1981; Hinton, 1981; Futuyma, 1990). With reference to chrysomelids, Klausnitzer and Forster (1971) reported SEM egg-chorion findings on four leaf beetle species none of them a chrysomeline. Mazzini (1974) described the egg-chorion ultrastructure of two Chrysomelinae, *Chrysolina grossa* and *Plagiodera versicolora*, while Futuyma (1990) compared this ultrastructure in six species of the North American galerucine genus *Ophraella*. In view of the scanty information on this subject and with the purpose of gaining some insight into the taxonomic and evolutionary interrelationships of Chrysomelinae, SEM analyses were undertook on various representative species belonging to this subfamily.

4.1 Egg-chorion ultrastructure in species of Chrysomelinae

Eggs laid by captive females or, in a few cases, dissected from inside the abdomens of recently killed

ones were collected. These eggs were carefully cleaned with distilled water using a fine paint-brush under a stereomicroscope. Then they were covered with a thin (300 Å) gold layer in vacuum to make them suitable for SEM observations and micrographs. The following 16 chrysomeline species from the geographical sources listed were analysed.

Timarchina	
Timarcha balearica Gory	Mallorca (Balearic Is., Spain)
Timarcha espanoli Bech.	Murcia (S.E. Spain)
Timarcha tenebricosa (F.)	Pyrenees of Lleida (Spain)
Chrysolinina	
Chrysolina americana (L.)	Mallorca (Balearic Is., Spain)
Chrysolina banksi (F.)	Mallorca (Balearic Is., Spain)
Chrysolina carnifex ssp. *melanaria* Suffr.	Herault (S. France)
Chrysolina fastuosa (Scop.)	Val d'Aran (Pyrenees, Spain)
Chrysolina graminis (L.)	Pyrenées Atlantiques (S. France)
Chrysolina peregrina (H.S.)	Mallorca (Balearic Is., Spain)
Chrysolina *polita* (L.)	Lleida (Catalonia, Spain)
Chrysolina viridana (Kust.)	Mallorca (Balearic Is., Spain)
Gonioctenina	
Gonioctena variabilis Ol.	Málaga (Andalucia, Spain)
Chrysomelina	
Colaspidema atrum (Olivier)	Barcelona (Catalonia, Spain)
Plagiodera versicolora Laich.	Barcelona (Catalonia, Spain)
Phaedon cochleariae (F.)	Barcelona (Catalonia, Spain)
Prasocuris junci (Brahm)	Barcelona (Catalonia, Spain)

As seen above although only four subtribes, Timarchina, Chrysolinina, Gonioctenina and Chrysomelina are represented in the sample, since several *Timarcha* and *Chrysolina* species are checked, the results provide a reliable information on the egg-chorion ultrastructures and their interrelationships among species of these two genera.

Four main types of ultrastructural elements are found in the egg-chorion of chrysomelines: a) reticles of polygonal shape, b) scales or warts, c) irregular comma- and dot-like reliefs, and d) fenestra, that is uniform punctures in the outer egg-chorion surface. These four types of sculpturing elements appear alone or can be combined in pairs depending on each species.

Timarcha. Eggs are laid in clusters in the ground, with or without faecal matter (Jolivet, 1948). Those of the three species sampled show a rather blunted oval outline and an orange-reddish colour, turning to yellow with age. Their sizes, measured from SEM micrographs, are correlated with the body lengths of their adults: *T. tenebricosa* has the largest eggs (3.85 mm length – 2.15 mm width), *T. espanoli* (3.75–2.15 mm) and *T. balearica* (2.90–1.45 mm). All these species have eggs bearing outer polygonal reticles made up of hexagons or pentagons without any clear differentiation of the inner part. The ridges of these reticles are rather thick and raised in *T. balearica* (Fig. 6), whereas they are very slightly raised in *T. tenebricosa* and *T. espanoli* (Fig. 7). The last two species are almost indistinguishable by their egg-chorions.

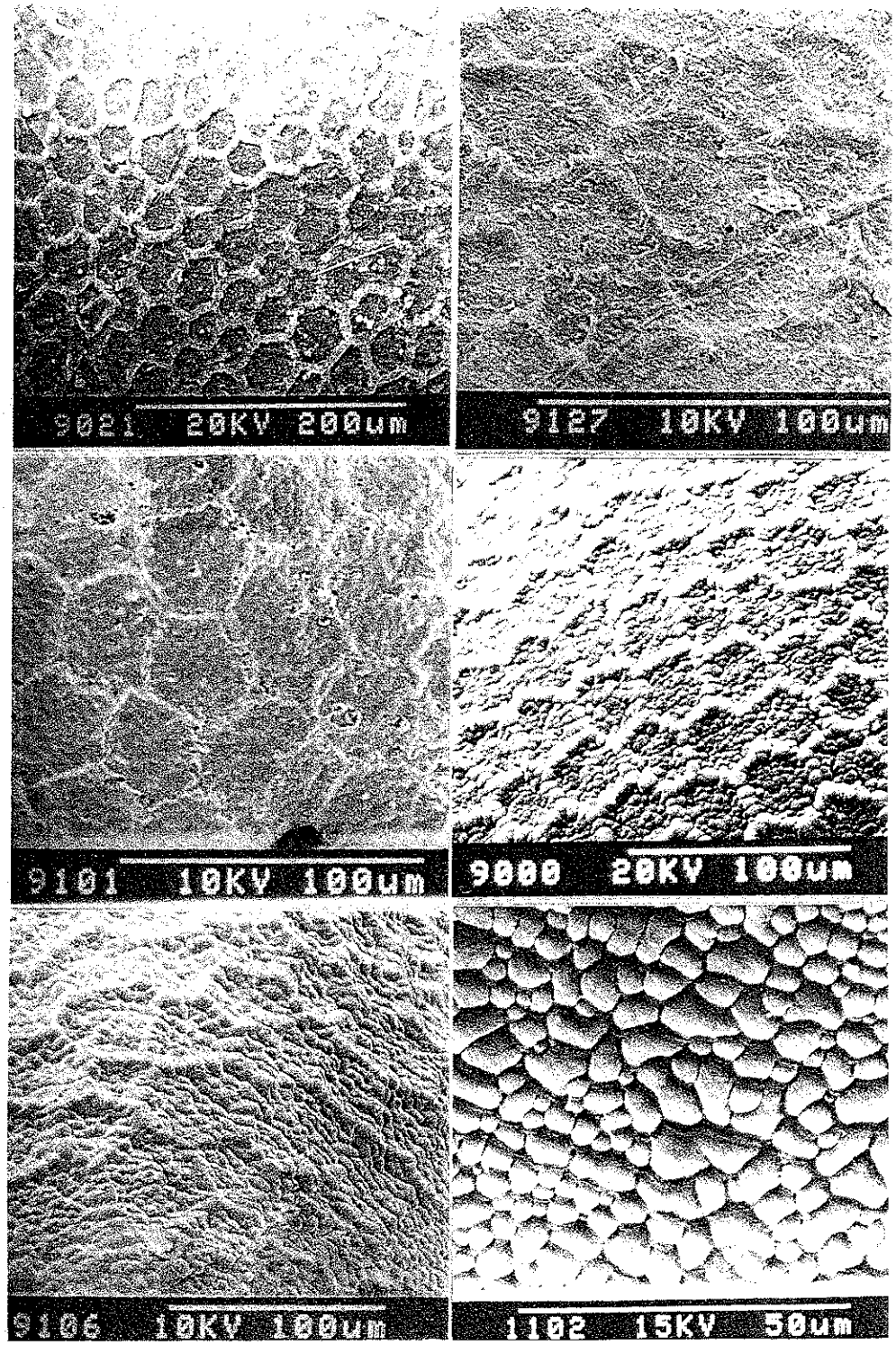
Chrysolina. In contrast to *Timarcha* the eight *Chrysolina* species examined are strikingly heterogeneous in gross external appearance as well as in their egg-chorion ultrastructure. Most have a length/width ratio close to 2 as in *Timarcha* but some, such as *C. graminis* and *C. viridana*, have ratios higher than 2.5, so that their eggs are much more elongated than the remainder. The eggs in these *Chrysolina* species range from 1.5 to 2.5 mm in length and from 0.5 to 1.0 mm in width. The prevalent colour of these eggs is greyish-brownish but some are yellow such as those of *C. viridana.* The egg-chorion pattern of *C. fastuosa* most closely resembling that of *Timarcha* because of its outer polygonal reticles. However, these are composed of thinner ridges than in *Timarcha* and show a slightly roughened inner differentiation (Fig. 8).

The egg surfaces of *C. viridana* and *C. graminis* also show outer reticles but they are made of the same scale elements as those found inside. The scales comprising the ridges in *C. viridana* are larger than those of the internal cells (Fig. 9) whilst in *C. graminis* both kinds of scales are of the same size and the reticles are a slightly less prominent (Fig. 10).

The chorion ultrastructure of *C. carnifex* is constituted by reticles made of thick and coarse walls which surround an irregular inner space filled with small warts (Fig. 13).

In the remaining *Chrysolina* species reticles are absent and the egg surface is made of wart-like or comma-like elements and/or punctures. *C. banksi* and *C. americana* exhibit a paved surface, the former bearing warts of different sizes and without punctures (Fig. 11) whereas the latter shows similar warts and a few scattered punctures (Fig. 12). The eggs of *C. polita* and *C. peregrina* have a chorion surface with other prevalent structural elements. Those of *C. polita* are completely covered with fenestrae whose diameters are similar to that of the interfenestral distance (Fig. 14). Those of *C. peregrina* display irregular comma-like and dot-like elements separated by a maze of lower background areas (Fig. 15).

In addition, the eggs of one Gonioctenina species, *Gonioctena variabilis*, are brownish, smaller and much more elongated (length 1.63 – width 0.58 mm, ratio 2.8) than those of most *Chrysolina.* Moreover, chorion ultrastructure resembles that of *C. peregrina* but their comma- and dot-like elements are sharper and somewhat more linked among themselves than in this species (Fig. 16). Of the four Chrysomelina species examined, *Plagiodera versicolora*, and *Phaedon cochleariae* lack any external egg-chorion

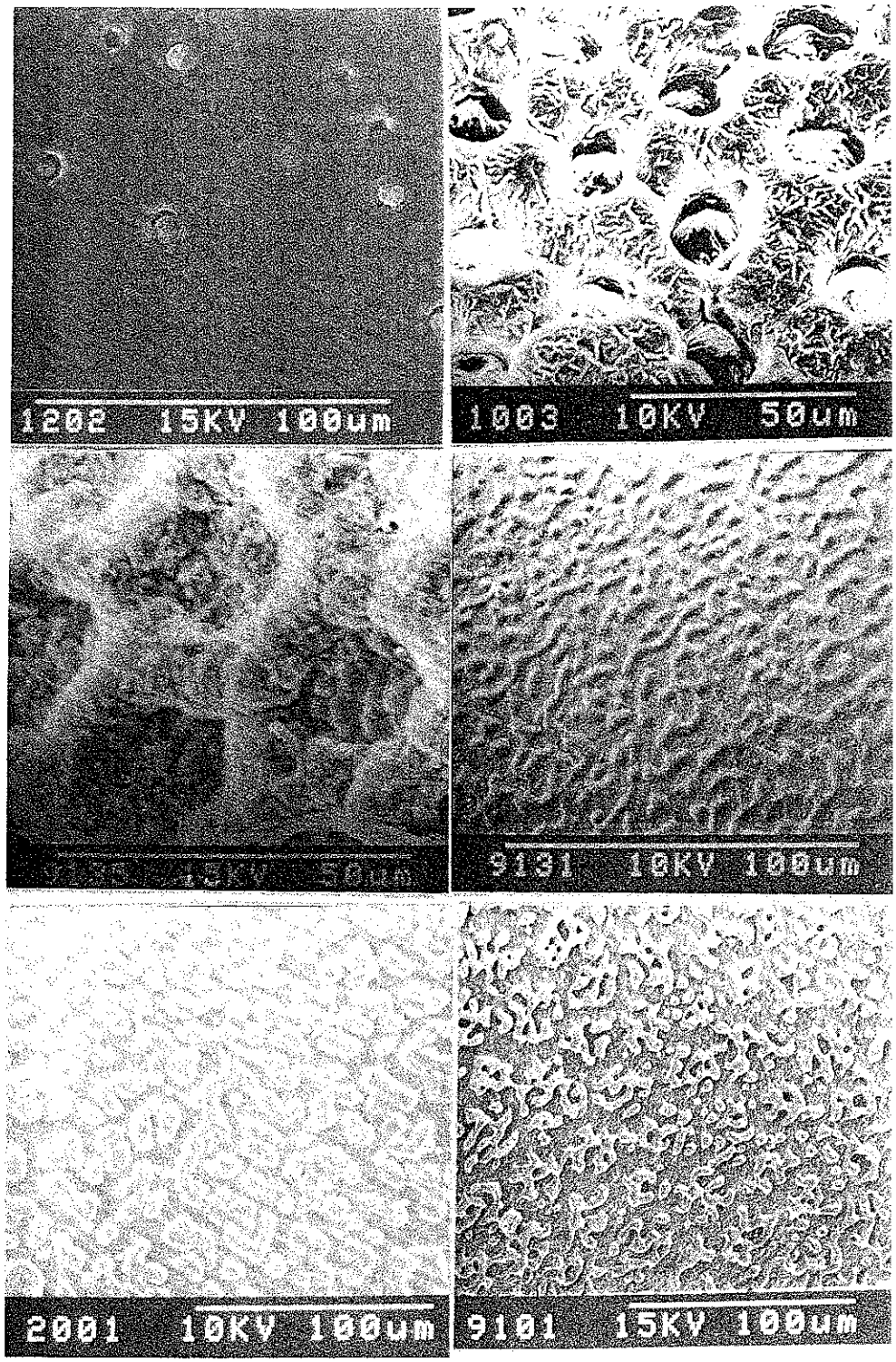


Figs. 6–11. Egg-chorion ultrastructure in Chrysomelinae. From upper left to bottom right: *Timarcha balearica*, *T. espanoli*, *Chrysolina fastuosa*, *C. viridana*, *C. graminis* and *C. banksi*.

ultrastructure, *Prasocuris junci* bears a paving sculpture of small wart elements and *Colaspidema atrum* exhibits an ultrastructure recalling those of *Chrysolina peregrina* and *Gonioctena variabilis* but made of much more irregular and complex reliefs (Fig. 17).

5. Discussion and conclusions

The use of three different characters in Chrysomelinae leaf beetles, two cytogenetic and one of egg ultrastructure, may provide valuable phylogenetic information because they have shown to be largely



Figs. 12–17. Egg-chorion ultrastructure in Chrysomelinae. From upper left to bottom right: *Chrysolina americana*, *C. polita*, *C. carnifex*, *C. peregrina*, *Gonioctena variabilis* and *Colaspidema atrum*.

independent of each other, and consequently are less likely to be subjected to misleading interpretations.

Timarchina, having *Timarcha* as its unique genus, comprises the most plesiomorphous species of Chrysomelinae in larval and adult morphology, male genitalia and some life history, features (Sharp & Muir, 1912; Chen, 1934; Jolivet, 1948; Iablokoff-Khnzorian, 1966; Cox, 1982; Secno & Wilcox, 1982; Daccordi, 1983; Mann & Crowson, 1983, 1984). Many taxa of *Timarcha* possess the ancestral karyotype, 2n=20 (Xyp), of Coleoptera Polyphaga, and their 1C genome sizes, of about 0.8–0.9 pg, are very

similar. Furthermore, their chorion ultrastructure is made of outer polygonal reticles with no clear differentiation of inner areas. Of seventeen chosen characters of phylogenetic value, three were apomorphic in *Timarcha* (Timarchina), whereas there were eleven in Chrysolinina and Doryphorina, and fifteen in Chrysomelina and Phyllodectina (Table 2).

Therefore, Timarchina are further apart from Chrysolinina/Doryphorina and Chrysomelina/Phyllodectina than these four subtribes are between each pair. This reinforces the current taxonomy of Timarchina as a unique and independent tribe, Timarchini, different from the other Chrysomelini, including all the additional Chrysomelinae (Seeno & Wilcox, 1982; Daccordi, 1983).

As indicated above, *Chrysolina* and *Oreina* species (Chrysolinina) are more advanced than those of *Timarcha*. Despite their wide range of chromosome

Table 2. Exophenotypic and endophenotypic characters of phylogenetic value in some subtribes of Chrysomelinae. P = plesiomorphous, A = apomorphous

	Timarchina	Chrysolinina/ Doryphorina	Chrysomelina/ Phyllodectina
1)	eggs laid on the ground (P)	eggs laid on the host plants (A)	eggs laid on the host plants (A)
2)	egg-chorion made of reticles without inner differentiation (P)	egg-chorion of other types (A)	egg-chorion of other types (A)
3)	larvae without tubercles (P)	larvae with tubercles (A)	larvae with tubercles (A)
4)	larvae without defensive glands (P)	larvae with one pair of glands (P)	larvae with many glands (A)
5)	larvae with 4-dentate mandibles (P)	larvae with 3-dentate mandibles (A)	larvae with 3-dentate mandibles (A)
6)	larvae with 7 abdominal spiracles (A)	larvae with 8 abdominal spiracles (P)	larvae with 8 abdominal spiracles (P)
7)	larvae with three instars (A)	larvae with four instars (P)	larvae with three instars (A)
8)	larvae having tarsal claws without an inner tooth (P)	larvae having tarsal claws usually with an inner tooth (A)	larvae having tarsal claws usually with an inner tooth (A)
9)	adult genitalia with ring-shaped tegmen (P)	adult genitalia with V-shaped tegmen (A)	adult genitalia with V-shaped tegmen (A)
10)	aedeagus with two basal struts (P)	aedeagus without basal struts (A)	aedeagus without basal struts (A)
11)	2nd. tarsomere similar to 3rd. (P)	2nd. tarsomere narrower than 3rd.	2nd. tarsomere narrower than 3rd
12)	mentum large (P)	mentum small (A)	mentum small (A)
13)	front coxal cavities closed (A)	front coxal cavities open (P)	front coxal cavities open (P)
14)	maxillary palpi broad, with apical sensilla (P)	maxillary palpi slender, with basal sensilla (A)	maxillary palpi slender with basal sensilla (A)
15)	ventral nerve cord with 7th. ganglion incompletely fused to 7+8 ganglion (P)	ventral nerve cord with 7th. ganglion completely fused to 7+8 ganglion (A)	ventral nerve cord with 7th ganglion completely fused to 7+8 ganglion (A)
16)	modal haploid number of 10 chromosomes (P)	modal haploid number of 12 but two spp. with 10 chromosomes (A)	modal haploid number of 17 chromosomes (A)
17)	average genome size 0.8–0.9 pg (P)	average genome size 0.8–1 pg (P)	average genome size mostly <0.5 pg (A)

numbers, with a highest value of 2n(♂)=47 chromosomes (Petitpierre, 1981), all but one species of *Chrysolina* and *Oreina* have diploid values higher than 20 chromosomes, the modal karyotype of *Timarcha*. *Oreina viridis* the exception to this rule, probably demonstrates that both Timarchina and Chrysolinina share an hypothetical common ancestor having an asymmetric karyotype of 20 chromosomes, made of large and small elements. The genome size of many species of *Chrysolina* and of one *Oreina* species (0.8–1.0 pg) resemble those found in *Timarcha* although some species (*C. banksi*) have seemingly undergone small or even dramatic (*C. carnifex*) increases and at least *C. americana, C. pyrenaica* and *C. viridana*, have shown certain decreases in genome size. These shifts in genome size may or may not correspond with changes in chromosome number. Moreover, the egg-chorion ultrastructures observed in *Chrysolina* species are strikingly heterogeneous and embody new elements such as scales, warts and fenestrae, unknown in *Timarcha*. Nevertheless, *C. fastuosa* displays an egg-chorion pattern somewhat similar to that of *Timarcha*, with an outer polygonal reticle of lineal ridges similar to those of *Timarcha* but differing in its incipient wart appearance of reticular inside. This pattern of *C. fastuosa* egg-chorion is probably transitional. On the other hand, the karyotype of *C. fastuosa* has n=12 chromosomes as in all the remaining *Chrysolina* with Lamiaceae as host plants (Jolivet & Petitpierre, 1976; Petitpierre, 1981; Petitpierre & Segarra, 1985).

C. viridana and *C. graminis* share very similar egg-chorion ultrastructures based on scale elements, but still keeping outer reticles built up of scale pieces instead of lineal ridges, as found in *Timarcha* and *C. fastuosa*. *C. viridana* and *C. graminis* are close morphologically, share 24 chromosomes, and feed mainly on *Mentha* spp. (Lamiaceae) (Petitpierre, 1988b; Bourdonné & Doguet, 1991). Their remarkable resemblance in egg-chorion ultrastructure gives additional support to their presumed strong genetic homology.

Another species, *Chrysolina grossa* studied by Mazzini (1974), has an egg-chorion resembling those of *C. viridana* and *C. graminis*, being made up of polygonal reticles each constituted by many small scales but having additionally some scattered holes interpreted as aeropili. This egg-chorion of *C. grossa* may be assumed as an intermediate step towards that of *C. americana* where the reticles have disappeared and some punctures are evident among the paved surface of wart-elements. A similar paved egg-chorion but without punctures is observed in *C. banksi*. All these species share karyotypes of 23 or 24 rather similar chromosomes and their feeding preferences are towards Lamiaceae (Petitpierre, 1983, 1988b). Nevertheless, their genome sizes do not provide any substantial agreement with their similarities in egg-chorion because *C. banksi* shows a DNA content two times that of *C. americana* and their external morphologies are strikingly divergent.

The most specialized egg-chorion surfaces are those of *C. polita, C. carnifex, C. peregrina, Gonioctena variabilis* and *Colaspidema atrum*. Fenestrae are the predominant elements in *C. polita*, rough and thick reticles are characteristic of *C. carnifex* whilst comma- or dot-like elements distinguish the egg surface of *C. peregrina, Gonioctena variabilis* and *Colaspidema atrum*. These egg-chorion features are neither correlated with peculiar karyotypes nor with specific genome sizes except for *C. carnifex*, a species with 2n=40 chromosomes (Petitpierre, 1981) and possessing the highest amount of haploid nuclear DNA found in the Coleoptera (3.69 pg). *C. polita* is a 24-chromosome species feeding on Lamiaceae as some of the previous species. *C. peregrina, Gonioctena variabilis* and *Colaspidema atrum* are widely divergent species, classified in three different subtribes. The first two species also differ in their karyotypes, possessing 2n=46 and 2n=24 chromosomes, respectively (Petitpierre *et al.*, 1988), and in their trophic selection of Apiaceae and Fabaceae, respectively (Jolivet & Petitpierre, 1976a, 1976b).

Finally, the genome size gives interesting clues to the evolution of Chrysomelinae since the species belonging to the most advanced subtribes, Chrysomelina and Phyllodectina, display on average smaller genomes of < 0.5 pg than those of the highly plesiomorphic subtribe Timarchina and those of the less apomorphic subtribe Chrysolinina, which mostly have genomes of 0.8–1.0 pg. Consequently, the origin of Chrysomelina and Phyllodectina has probably taken place by decreases of genome size which are also documented in many groups of animals coupled to specialization (Hinegardner, 1976).

However, this trend is by no means unique since substantial increases of genome size are also reported in some *Chrysolina* species. The decreases in genome size found in Chrysomelina species may have an adaptive significance as a means of speeding up their development. This may result in bivoltine or multivoltine life cycles, contrary to the prevalent univoltine cycles of Timarchina and Chrysolinina. At least two, *Plagiodera versicolora* and *Phaedon cochleariae*, of the Chrysomelina, share egg-chorions almost devoid of any apparent ultrastructure which can be either interpreted as an apomorphic character and/or a feature related to the short incubation period of egg developmental stage when there is not a necessity for thicker external protective devices. This may be in part because of their fast embryonic development and

partly to the possibly great value of defensive substances accumulated in these eggs.

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References

Ado, N. Y., Petrov, N. B. & Filippovich, Y. B., 1985. Repeated and unique DNA sequences of some Lepidoptera species. J. Mol. Biochem. Physiol. 21:65–70.

Alvarez-Fuster, A., Juan, C. & Petitpierre, E., 1991. Genome size in *Tribolium* flour-beetles: inter- and intraspecific variation. Genet. Res., Camb. 58:1–5.

Belda, J. E., Cabrero, J., Camacho, J. P. M. & Rufas, J. S., 1991. Role of C-heterochromatin in variation of nuclear DNA amount in the genus *Chorthippus* (Orthoptera, Acrididae). Cytobios 67:13–21.

Bennett, M. D., 1985. Intraspecific variation in DNA amount and its nucleotypic dimension in plant genetics. In: Plant Genetics. Alan R. Liss, pp. 283–302.

Bennett, M. D. & Smith, J. B., 1976. Nuclear DNA amounts in angiosperms. Phil. Trans. R. Soc. Lond. B 274:227–274.

Bennett, M. D., Smith, J. B. & Heslop-Harrison, J. S., 1982. Nuclear DNA amounts in angiosperms. Proc. R. Soc. Lond. B216:179–199.

Biemont, J. C., Chauvin, G. & Hamon, C., 1981. Ultrastructure and resistance to water loss in eggs of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). J. Insect Physiol. 27:667–679.

Bier, K. & Müller, W., 1969. DNA-Messungen bei insekten und eine hypothesis über retardierte evolution und bnesonderen DNS-reichtum im Tierreich. Biol. Zbl. 88:425–449.

Black, C. W. IV & Rai, K. S., 1988. Genome evolution in mosquitoes: intraspecific and interspecific variation in repetitive DNA amounts and organization. Genet. Res., Camb. 51:185–196.

Bosch, R., 1989. Contenido de DNA nuclear en especies baleares de Scarabaeoidea (Coleoptera) y su contribución a la filogenia del grupo. Tesina de Licenciatura, Univ. Balears, Palma de Mallorca, 80 pp.

Bourdonné, J. C. & Doguet, S., 1991. Données sur la biosystématique des *Chrysolina l.s.* (Coleoptera: Chrysomelidae: Chrysomelinae). Ann. Soc. ent. Fr. (N.S.) 27:29–64.

Cavalier-Smith, T., 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J. Cell Sci. 34:247–278.

Cavalier-Smith, T., 1985. Introduction: the evolutionary significance of genome size. In: T. Cavalier-Smith (ed.) The evolution of genome size. John Wiley, pp. 1–36.

Chen, S., 1934. Sur la position systématique du genre *Timarcha* Latr. Bull. Soc. ent. Fr. 39:35–38.

Cox, M. L., 1982. Larvae of the British genera of chrysomeline beetles (Coleoptera, Chrysomelidae). Syst. Entomol. 7:297–310.

Crowson, R. A., 1981. Biology of the Coleoptera. Academic Press.

Daccordi, M., 1983. Quelques réflexions sur la distribution de Chrysomelinae (Col. Chrysomelidae). Bull. Soc. ent. Fr. 88:448–451.

Fox, D. P., 1972. The DNA content of related species. Chromosomes Today 3:32–37.

Futuyma, D. J., 1990. Observations on the taxonomy and natural history of *Ophraella* Wilcox (Coleoptera: Chrysomelidae), with description of a new species. J. New York Entomol. Soc. 98:163–186.

Gold, J. R. & Amemiya, C. T., 1987. Genome size in North American minnows (Cyprinidae). II. Variation among 20 species. Genome 29:481–489.

Greilhuber, J. & Speta, F., 1985. Geographical variation of genome size at low taxonomic levels in the *Scilla bifolia* alliance (Hyacinthaceae). Flora 176:434–438.

Hewitt, G. M., 1979. Orthoptera. In: B. John (ed) Animal Cytogenetics vol. 3 Insecta 1, Gebrüder Borntraeger, Berlin.

Hinegardner, R., 1973. Cellular DNA content of the Mollusca. Comp. Biochem. Physiol. 47A:447–460.

Hinegardner, R., 1976. Evolution of genome size. In: F. J. Ayala (ed.) Molecular Evolution, Sinauer Assoc., Massachussets, pp. 179–199.

Hinton, H. E., 1981. Biology of insect eggs. 3 vols., Pergamon Press, Oxford.

Horner, H. A. & Macgregor, H. C., 1983. C value and cell volume: their significance in the evolution and development of amphibians. J. Cell Sci. 63:135–146.

Iablokoff-Khnzorian, S. M., 1966. Considérations sur l'édéage des Chrysomelidae et son importance phylogénique. L'Entomologiste 22:115–138.

John, B. & Hewitt, G. M., 1966. Karyotype stability and DNA variability in the Acrididae. Chromosoma 20:155–172.

John, B. & Miklos, G., 1988. The eukaryote genome in development and evolution. Allen & Unwin, London.

Jolivet, P., 1948. Introduction à la biologie des *Timarcha* (Col. Chrysomelidae). Misc. Ent. 45:1–32.

Jolivet, P. & Petitpierre, E., 1976a. Sélection trophique et évolution chromosomique chez les Chrysomelinae. Acta Zool. Pathol. Antwerp. 66:59–90.

Jolivet, P. & Petitpierre, E., 1976b. Les plantes-hôtes connues des *Chrysolina* (Col. Chrys.). Essai sur les types de sélection trophique. Ann. Soc. ent. Fr. (N.S.) 12:123–149.

Jost, E. & Mameli, M., 1972. DNA content in nine species of Nematocera with special reference to the sibling species of the *Anopheles maculipennis* group and the *Culex pipiens* group. Chromosoma 37:201–208.

Juan, C. & Petitpierre, E., 1991. Evolution of genome size in darkling beetles (Coleoptera, Tenebrionidae). Genome 34:169–173.

Klausnitzer, B. & Forster, G., 1971. Zur eimorphologie einiger mitteleuropäischer Chrysomelidae (Coleoptera). Polskie Pismo Ent. 41:428–437.

Laird, C. D., 1973. DNA of *Drosophila* chromosomes. Ann. Rev. Genet. 7:177–204.

Larson, A., 1984. Neotological inferences of evolutionary pattern and process in the salamander family Plethodontidae. Evolutionary Biology 17:119–217.

Laurie, D. A. & Bennett, M. D., 1985. Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation. Heredity 55:307–313.

Levin, D. A. & Fundenburg, S. W., 1979. Genome size in Angiosperms: temperate versus tropical species. Am. Nat. 114:784–795.

Lewin, R., 1990. Genes IV. J. Wiley & Sons, New York.

Mann, J. S. & Crowson, R. A., 1983. Phylogenetic significances of the ventral nerve cord in the Chrysomeloidea (Coleoptera: Phytophaga). Syst. Entomol. 8:103–119.

Mann, J. S. & Crowson, R. A., 1984. On the digitiform sensilla of adult leaf beetles (Coleoptera; Chrysomelidae). Entomol. Gener. 9:121–133.

Mazzini, M., 1974. Sulla fine structura del micropilo negli insetti. Redia 55:343–372.

Mazzini, M., 1975. Sulla fine structura del micropilo negli insetti. II. *Adalia bipunctata*. Redia 185–191.

McLaren, I. A., Sevigny, J. M. & Frost, B. W., 1989. Evolutionary and ecological significance of genome sizes in the copepod genus *Pseudocalanus*. Can. J. Zool. 67:565–569.

Olmo, E., 1983. Nucleotype and cell size in vertebrates: a review. Basic and Applied Histochem. 27:227–256.

Pasteels, J. M., Braekman, J. C. & Daloze, D., 1988. Chemical defense in the Chrysomelidae. In: P. Jolivet, E. Petitpierre and T. H. Hsiao (eds.), Biology of Chrysomelidae. Kluwer Acad. Publ., Dordrecht, pp. 233–252.

Peterson, N. F., 1931. The bionomics and comparative morphology of the early stages of certain Chrysomelidae (Col. Phyt.). Proc. Zool. Soc. Lond. 4:879–940.

Petitpierre, E., 1970. Cytotaxonomy and evolution of *Timarcha* Latr., (Col. Chrysomelidae). Genét. Ibér. 22:67–120.

Petitpierre, E., 1976. Further cytotaxonomical and evolutionary studies on the genus *Timarcha* Latr. (Col. Chrysomelidae). Genét. Ibér. 28:58–72.

Petitpierre, E., 1981. New data on the cytology of *Chrysolina* (Mots.) and *Oreina* Chevr. (Coleoptera, Chrysomelidae). Genetica 54:265–272.

Petitpierre, E., 1982. Chromosomal findings on 22 species of Chrysomelinae (Chrysomelidae: Coleoptera). Chrom. Infor. Serv. 32:22–23.

Petitpierre, E., 1983. Karyometric differences among nine species of the genus *Chrysolina* (Coleoptera, Chrysomelidae). Can. J. Genet. Cytol. 25:33–39.

Petitpierre, E., 1988a. Cytogenetics, cytotaxonomy and genetics of Chrysomelidae. In: P. Jolivet, E. Petitpierre and T. H. Hsiao (eds.), Biology of Chrysomelidae. Kluwer Acad. Publ., Dordrecht, pp. 131–160.

Petitpierre, E., 1988b. A new contribution to the cytogenetics and cytotaxonomy of the Chrysomelinae (Coleoptera, Chrysomelidae). Cytobios 54:153–159.

Petitpierre, E. & Segarra, C., 1985. Chromosomal variability and evolution of Chrysomelidae (Coleoptera) particularly that of Chrysomelinae and Palearctic Alticinae. Entomography 3:403–425.

Petitpierre, E., Segarra, C., Yadav, J. S. & Virkki, N., 1988. Chromosome numbers and meioformulae of Chrysomelidae. In: P. Jolivet, E. Petitpierre and T. H. Hsiao (eds.), Biology of Chrysomelidae. Kluwer Acad. Publ., Dordrecht, pp. 161–186.

Petitpierre, E., Juan, C., Fujiyama, S. & Carreras, I., 1991. Las especies gemelas del complejo *Chrysolina aurichalcea* Mann. (Coleoptera: Chrysomelidae): divergencia genética y evolución. XXVI Jorn. Luso-Espanholas Genét., Coimbra, p. 96.

Petitpierre, E., Segarra, C. & Juan, C. (in press). Genome size and chromosomal evolution in leaf beetles (Coleoptera, Chrysomelidae). Hereditas.

Price, H. J., Bachmann, K., Chambers, K. L. & Rigs, J., 1980. Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. Bot. gaz. 141:195–198.

Regland, C. J. & Gold, J. R., 1989. Genome size variation in the North American sunfish genus *Lepomis* (Pisces: Centrarchidae). Genet. Res., Camb. 53:173–182.

Rao, P. N. & Rai, K. S., 1987. Inter and intraspecific variation in nuclear DNA content in *Aedes* mosquitoes. Heredity 59:253–258.

Rasch. E. M., Barr, H. J. & Rasch, R. W., 1971. The DNA content of sperm of *Drosophila melanogaster*. Chromosoma 33:1–18.

Rayburn, A. L., Price, H. J., Smith, J. D. & Gold, J. R., 1985. C-band heterochromatin and DNA content in *Zea mays*. Amer. J. Bot. 72:1610–1617.

Rees, H., Shaw, D. D. & Wilkinson, P., 1978. Nuclear DNA variation among acridid grasshoppers. Proc. R. Soc. Lond. B 202:517–525.

Sakanoue, S. & Fujiyama, S., 1987. Allozymic variation among geographic populations of *Chrysolina aurichalcea* (Coleoptera, Chrysomelidae). Kontyû, Tokyo 55:437–449.

Samols, D. & Swift, H., 1979. Genome organization in the fleshfly *Sarcophaga bullata*. Chromosoma 75:129–143.

Seeno, T. N. & Wilcox, J. A., 1982. Leaf Beetle Genera (Coleoptera: Chrysomelidae). Entomography 1:1–221.

Sharp, W. E. & Muir, F., 1912. The comparative anatomy of the male genital tube in Coleoptera. Trans. ent. Soc. Lond. 3:477–642.

Sherwood, S. W. & Patton, J. L., 1982. Genome evolution in pocket gophers (genus *Thomomys*) II. Variation in cellular DNA content. Chromosoma 85:163–179.

Sparrow, A. H., Price, H. J. & Underbrink, A. G., 1972. A survey of DNA content per cell and per chromosome of prokaryotic and eukaryotic organisms: some evolutionary considerations. In: H. H. Smith (ed.) Evolution of genetic systems, Brookhaven Symp. Quant. Biol., pp. 451–494.

Szarski, H., 1976. Cell size and nuclear DNA content in vertebrates. Inter. Rev. Cytol. 44:93–111.

Szarski, H., 1983. Cell size and the concept of wasteful and frugal evolutionary strategies. J. Theor. Biol. 105:201–209.

Takizawa, H., 1976. Larvae of the genus *Gonioctena* Chevrolat (Coleoptera, Chrysomelidae): descriptions of Japanese species and the implication of larval characters for the phylogeny. Kontyû, Tokyo 44:444–468.

Vidal, O. R., 1984. Chromosome numbers of Coleoptera from Argentina. Genetica 65:235–239.

Virkki, N., 1964. On the cytology of some Neotropical chrysomelids Ann. Acad. Sci. Fenn. AIV 54:1–22.

Watson, J. D., Hopkins, N. H., Roberts, J. W., Steitz, J. A. & Weiner, A. M., 1987. Molecular biology of the gene 4th ed. The Benjamin/Cummings Publ. Co., Menlo Park.

Zacharias, H., 1979. Underreplication of a polytene chromosome arm in the chironomid *Prodiamesa olivacea*. Chromosoma 72:27–51.

Zacharias, H., Hennig, W. & Leoncini, O., 1982. Microspectrophotometric comparison of the genome sizes of *Drosophila hydei* and some related species. Genetica 58:153–157.