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# Preliminary data on the chromosomes of European Alticinae

## (Coleoptera, Chrysomelidae)

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### Abstract

The number of chromosomes and, in several cases, the sex-determining systems have been analyzed in fourteen species of European Alticinae. *Altica lythri* has $11^{II} + X + y$, *Chaetocnema conducta* $10^{II} + Xy_p$, *Chaetocnema chlorophana* $9^{II} + Xy_p$, *Chaetocnema tibialis* $10^{II} + Xy_r$, *Aphthona herbigrada* and *Aphthona illigeri* $13^{II} + Xy$, *Aphthona nigriceps* $2n = 32$, *Aphthona cyparissiae* $15^{II} + Xy$, *Longitarsus pratensis* $13^{II}$, *Longitarsus tabidus* $13^{II} + Xy$, *Longitarsus obliteratoides* $15^{II}$, *Psylliodes chrysocephala* $17^{II} + Xy_p$, *Podagrica menetriesi* $19^{II} + Xy_p$, and *Sphaeroderma testaceum* $25^{II} + Xy$. The spermatogonial metaphases or metaphases II obtained in *Aphthona nigriceps, Longitarsus tabidus* and *Podagrica menetriesi* have shown complements made of medium or small metacentric chromosomes mostly. The range of variation in the number of chromosomes is wide as it is usual for many chrysomelids. Starting from the assumed primitive karyotype of Alticinae, $11^{II} + Xy_p$, the chromosomal evolution has proceeded by decreases of number in *Chaetocnema* but by several steps of increases in all the remaining genera except in *Altica*, probably due to centric fusions and fissions, respectively. The chromosome number of *Sphaeroderma testaceum* $2n = 52$, is the highest found in Alticinae. The value of the tribes, Alticini, Chaetocnemini, Psylliodini, Podagricini and Sphaerodermini is partly supported by the present chromosomal findings. On the contrary, Longitarsini and Aphthonini tribes show a similar range of chromosome numbers. However, much more species should be analyzed before setting up final conclusions on the phylogenetics and systematics of Alticinae.

### Introduction

The formation of a new species is a process which implies a change in genetic constitution from a pre-existing ancestor species. This change occurs within a population since species can not be treated as a mere sum of independent individuals, they are organised in populations whose members belong to a common reproductive lineage. The crucial stage in the process of speciation is the acquisition of mechanisms of genetic isolation, preserving the new species against breeding with closely related organisms. There is not still a general agreement about the requirements to reach the reproductive isolation. Most population geneticists explain speciation as a gradual change in the pool of genes, being the reproductive isolation a by-product of this change. Through this way the accumulation of

point mutations would be sufficient to come in a new species, under the same general principles that govern changes in genic frequencies of conspecific populations (DOBZHANSKY, 1951; MAYR, 1963).

The recent use of electrophoretic techniques (HUBBY and LEWONTIN, 1966; LEWONTIN and HUBBY, 1966) has provided a valuable tool for measuring the degree of genetic divergence between taxonomically related species. From the results obtained in many groups of animals it can be presently concluded that a few genic substitutions may sometimes cause reproductive isolation. To become to a new species is not necessary to suffer a "genetic revolution", by replacement of a major part of the genes in an original genotype as some evolutionists have claimed in the past (see WHITE, 1978). The level of genetic divergence is usually in correspondence with that of morphological divergence, but there are some exceptions in the groups of rapid speciation as in five relict species of the pupfish *Cyprinodon* from Death Valley in California, where striking differences in morphology, ecology and behaviour are accompanied by very sligth genetic alterations (TURNER, 1974).

The role of chromosomal changes in speciation has been strongly supported in the recent years after the multiple cytogenetic analyses carried out on a large number of allied species. Most speciation events, about 98% according to WHITE (1978), are coupled with chromosomal rearrangements which undoubtedly supply an essential part in making the genetic characteristics of any species. The study of karyotype can be performed through six progressively higher levels of knowledge named from **alpha** to **zeta** karyology (WHITE, 1978). The most elemental level is represented by the **alpha** karyology, determining chromosome numbers and aproximate sizes only. Then follows the **beta** karyology where chromosomes and lengths of chromosomal arms are carefully measured. The great majority of karyological results are obtained on these two levels of analysis. However it is frequent the need of using other levels of karyology to get a clear differentiation between very similar karyotypes by banding procedures for instance, as it has been extensively employed in the karyology of Mammals during the recent years.

The value of karyotype can therefore be of high interest in cytotaxonomy and evolution for almost all groups of related species. When such a group of species is deeply worked out the different chromosomal rearrangements involved in its divergence could be identified and their possible order of occurrence established. That is, the phylogenetic series of changes which have been taken place from the ancestral to the most evolved species. Nevertheless, it should be remarked that karyological findings may not be worth in trying to detect more accurate interrelationships when higher taxonomic categories than genus are dealt with. If related genera or tribes show a similar range of variability or they are basically uniform, the chromosomal results shall be used with caution to avoid pure speculations.

Since many groups of animals can not be reared at laboratory without overcoming a great number of difficulties, both for their special requirements and/or because of their long biological cycles, it seems evident that they are not good materials for genetic studies. Nevertheless, the cytogenetic analysis can be carried out in any group of animals or plants and chromosomal findings should usually provide valuable information as we have appointed above.

The chromosome numbers of Chysomelidae are the most heterogeneous among the different families of beetles. Even some genera as for instance *Timarcha* and *Chrysolina* show a wide range of variation from $2n = 20$ to $2n = 44$, and $2n = 22$ to $2n = 48$, respecti-

vely PETITPIERRE, 1975; 1976; 1981). In particular, the cytogenetics of Alticinae has been largely examined in several papers by VIRKKI (see SMITH and VIRKKI, 1978 for references) on the Neotropical species. These papers are mainly concerned with chromosome structure and behaviour in meiosis, and on the cytotaxonomy and evolution of flea-beetles secondarily. It is especially noteworthy the finding of very large asynaptic sex-chromosomes in the subtribe Oedionychina, a feature which seems unique within Coleoptera (SMITH and VIRKKI, 1978).

VIRKKI (1970) assumed the formula $11^{II} + Xy_p$, found in *Forsterita* sp., as the primitive karyotype of Alticinae. The diversification from this karyotype has proceeded by both increases and decreases of chromosome number, and changes in sex-chromosome systems from the primitive "parachute" type $Xy_p$. The shifts in the number of chromosomes in Alticinae have also been remarkable as it is obvious from the extreme numbers of $2n = 8$ and $2n = 50$ (SMITH and VIRKKI, 1978). Particular emphasis should be given to the correlation between karyological evolution and the decrease in the number of spermatozoa per bundle, due to a reduction in the number of definitive mitosis forming spermatozoa (VIRKKI, 1969; 1970). On the other hand, this trend is not unique for Alticinae because it has also been observed in other Coleoptera (SMITH and VIRKKI, 1978).

Since karyological researches on European Alticinae have not been so far developed, we have started analysis with the aim to fill this gap of information for a better understanding of evolutionary interrelationships of this group.

TABLE I

| Species | Locality | no. ind. | no. cells |
|---|---|---|---|
| Altica lythri Aubè | Colomers (G.) La Riba, Pónt d'Armentera (T.) | 4 | 43 |
| Chaetocnema conducta Motsch. | Sils (G.) | 3 | 32 |
| Chaetocnema tibialis Illig. | Gavá (B.) Massanet (G.) | 4 | 46 |
| Chaetocnema chlorophana Duft. | Gavá (B.) | 5 | 74 |
| Aphthona illigeri Bedel | Esplugues, Garraf, La Garriga (B.) | 7 | 67 |
| Aphthona herbigrada Curtis | Vidrá (G.) | 3 | 30 |
| Aphthona cyparissiae Koch | Bordils (G.) | 1 | 18 |
| Aphthona nigriceps Redtb. | Gavá (B.) | 2 | 6 |
| Longitarsus pratensis Panz. | Esplugues, La Garriga (B.) Vidrá (G.) | 4 | 49 |
| Longitarsus tabidus Fabr. | La Garriga, Aiguafreda (B.) | 2 | 31 |
| Longitarsus obliteratoides Gruev | Tagamanent (B.) | 2 | 23 |
| Psylliodes chrysocephala L. | La Garriga (B.) | 1 | 2 |
| Podagrica menetriesi Fald. | Gavá (B.) | 4 | 40 |
| Sphaeroderma testaceum Fabr. | Bordils (G.) | 6 | 46 |

(B.)=BARCELONA; (T.)=TARRAGONA; (G.)=GERONA. These three provinces are in Catalonia, Northeastern of Spain.

TABLE II

Chromosomal data on fourteen species of European Alticinae

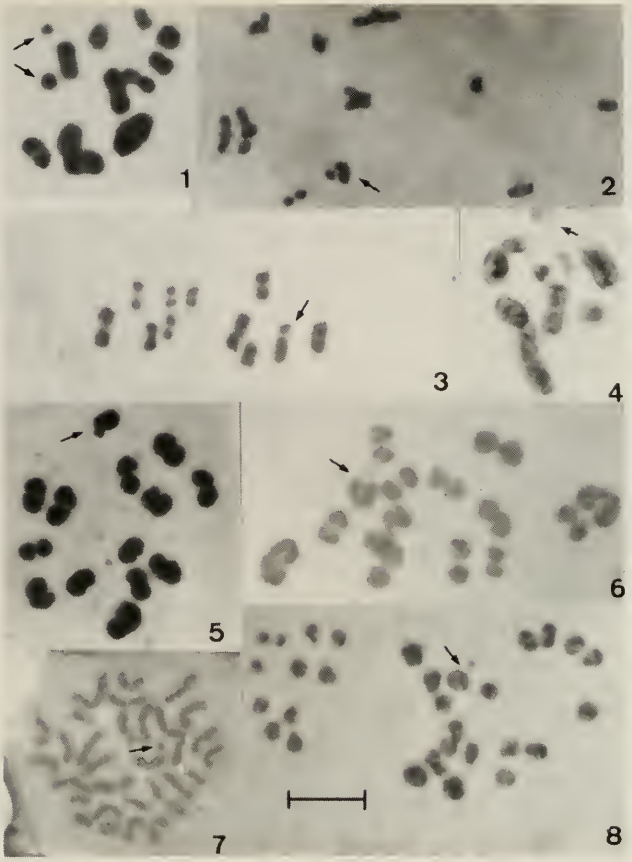
| Tribe/Species | 2n (♂) | Karyotypic formula |
|---|---|---|
| Alticini | | |
| Altica lythri Aubé | -- | $11^{II}$ + X + y |
| Chaetocnemini | | |
| Chaetocnema conducta Motsch. | -- | $10^{II}$ + $Xy_p$ |
| Chaetocnema tibialis Illig. | | $10^{II}$ + $Xy_r$ |
| Chaetocnema chlorophana Duft. | | $9^{II}$ + $Xy_p$ |
| Aphthonini | | |
| Aphthona illigeri Bedel | -- | $13^{II}$ + Xy |
| Aphthona herbigrada Curtis | -- | $13^{II}$ + Xy |
| Aphthona cyparissiae Koch | -- | $15^{II}$ + Xy |
| Aphthona nigriceps Redtb. | 32 | |
| Longitarsini | | |
| Longitarsus pratensis Panz. | -- | $13^{II}$ |
| Longitarsus tabidus Fabr. | -- | $13^{II}$ + Xy |
| Longitarsus obliteratoides Gruev | -- | $15^{II}$ |
| Psylliodini | | |
| Psylliodes chrysocephala L. | -- | $17^{II}$ + $Xy_p$ |
| Podagricini | | |
| Podagrica menetriesi Fald. | -- | $19^{II}$ + $Xy_p$ |
| Sphaerodermini | | |
| Sphaeroderma testaceum Fabr. | -- | $25^{II}$ + Xy |

## Material and Methods

The different species, their geographical sources, the number of individuals examined and cells counted are given in Table I. Only recently captured individuals were used for chromosomal analysis. After dissection, the testis were transferred on a clean slide covered with some drops of acetic orcein (from GURR's) for a 15 minutes of staining and a subsequent squashing. The best chromosome spreads were finally photographed by a Zeiss Photomicroscope and the micrographs were enlarged at ×2000.

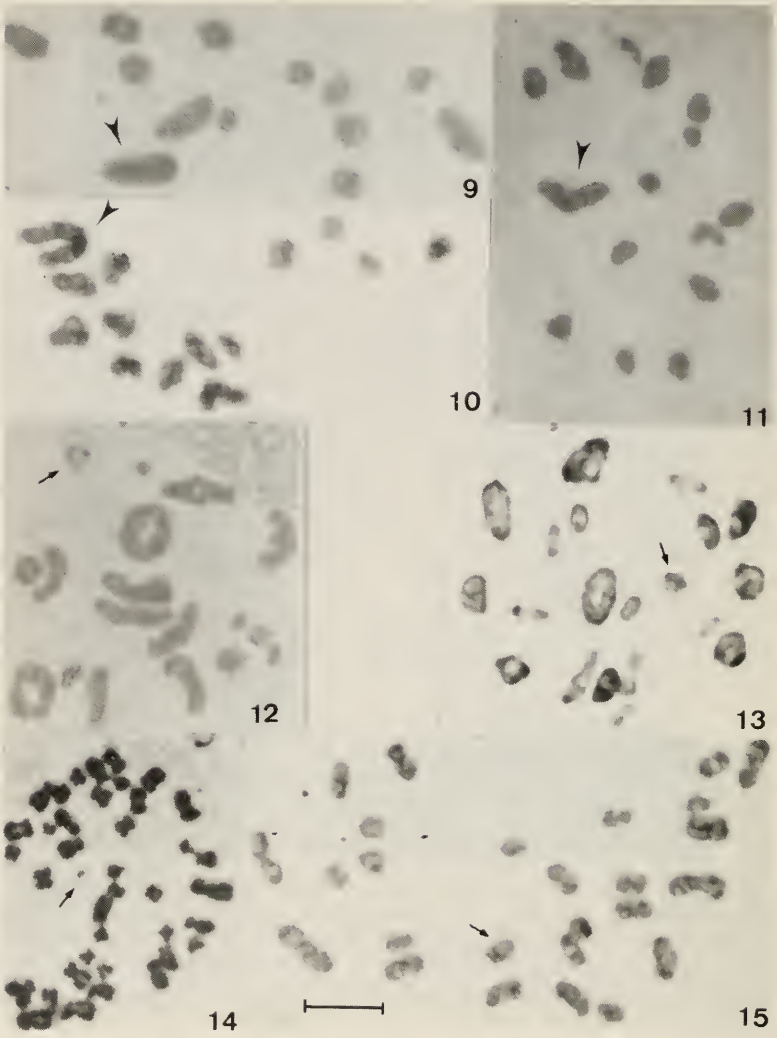
## Results

Chromosome observations (see Table II) were mainly performed on metaphases I but in few cases metaphases II and spermatogonial metaphases were also examined. A detailed observation of most metaphases I has made possible to distinguish the sex-bivalent and, in several cases, the type of association between both male sex-chromosomes. A di-

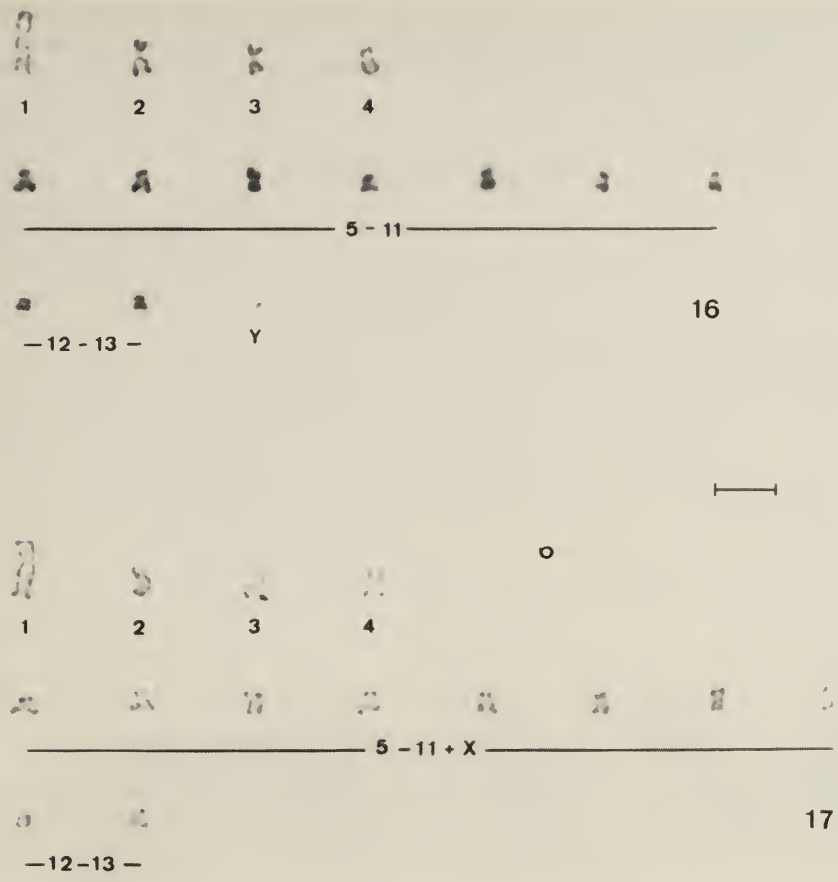



Figs. 1–8. Metaphases I of: *Altica lythri*, $11^{II} + X + y$ (Fig. 1); *Chaetocnema conducta*, $10^{II} + Xy_p$ (Fig. 2); *Ch. tibialis*, $10^{II} + Xy_r$ (Fig. 3); *Ch. chlorophana* $9^{II} + Xy_p$ (Fig. 4); *Aphthona herbigrada*, $13^{II} + Xy$ (Fig. 5); and *A. illigeri*, $13^{II} + Xy$ (Fig. 6). Spermatogonial metaphase of *Aphthona nigriceps* with $2n = 32$ (Fig. 7). The dot-like y-chromosome is indicated by an arrow. The chromosomes of each bivalent have just segregated in the anaphase I of *Aphthona cyparissiae*, $15^{II} + Xy$ (Fig. 8). The sex-bivalent is distinguished by an arrow. The bar is 5$\mu$m.

stance coorientated sex-chromosomes were observed in *Altica lythri* (Fig. 1). The "parachute" type of sex-bivalent, $Xy_p$, was found in *Chaetocnema conducta* (Fig. 2), *Chaetocnema chlorophana* (Fig. 4), *Psylliodes chrysocephala* (Fig. 12) and *Podagrica menetriesi* (Fig. 13). A rod type of sex-bivalent, $Xy_r$, was clearly apparent in *Chaetocnema tibialis* (Fig. 3). *Aphthona herbigrada* (Fig. 5), *A. illigeri* (Fig. 6), *A. cyparissiae* (Fig. 8) and *Sphaeroderma testaceum* (Fig. 15) have sex-bivalents, Xy, which seems to be connected by a chiasma though this point is not completely claryfied yet. In addition, the identification of the sex-bivalent has not been possible in some metaphases I such those of *Longitarsus: L. pratensis* (Fig. 9), *L. tabidus* (Fig. 10) and *L. obliteratoides* (Fig. 11), where the association between both sex chromosomes should also be chiasmatic.




Figs. 8–15. Metaphases I of: *Longitarsus pratensis*, $13^{II}$ (Fig. 9); *L. tabidus*, $14^{II}$ (Fig. 10); *L. obliteratoides*, $15^{II}$ (Fig. 11); *Psylliodes chrysocephala*, $17^{II} + Xy_p$ (Fig. 12); *Podagrica menetriesi* $19^{II} + Xy_p$ (Fig. 13); and *Sphaeroderma testaceum*, $25^{II} + Xy$ (Fig. 15). The largest autosomal bivalent of the three *Longitarsus* is arrowheaded. The sex-bivalent is pointed by an arrow. Spermatogonial metaphase of *Podagrica menetriesi* with $2n = 40$ (Fig. 14). The dot-like y-chromosome is indicated by an arrow. The bar is 5 $\mu$m.




Figs. 16–17. Haploid karyograms of *Longitarsus tabidus:* y-class (Fig. 16) and X-class (Fig. 17). The bar is 5 $\mu$m.

The autosomal bivalents of the three previous *Aphthona* (Figs. 5, 6 and 8) are of roughly similar sizes, while those of the remaining species could be classified in two or three groups of size (Figs. 1–4, 9–15). It is particularly noteworthy the existence of a large size bivalent in the three analyzed *Longitarsus* (Figs. 9–11).

We have analyzed spermatogonial mitosis from two species only: *Aphthona nigriceps* with 2n = 32 (Fig. 7) and *Podagrica menetriesi* with 2n = 40 (Fig. 14). The chromosomes of both species seem to be medium or small size metacentrics, and a few submetacentrics. The y-chromosome of *Aphthona nigriceps* and *Podagrica menetriesi* are dot-like elements.

Some metaphases II were obtained in *Longitarsus tabidus* which allow to make both classes of karyograms, including X or y-chromosome (Figs.16–17).The chromosomes are

metacentrics and among them stands out the first autosome having a very prominent large size, and the dot-like y-chromosome, the smallest of the karyogram. This autosome of large size should correspond to the largest bivalent in metaphase I (Fig. 10).

## Discussion and conclusions

Our chromosomal survey on fourteen species of European Alticinae should be considered as a preliminary report since only some genera have been available for analysis, and much more species should be checked of these genera dealt with here. However, seven different genera and tribes are included in the present report and five of them for the first time which allows to develop certain comparisons and conclusions.

Assuming the karyotype $11 + Xy_p$ as the most primitive for Alticinae (Virkki, 1970), that of *Altica lythri*, $11 + X + y$, is very similar and differs from the former by the asynaptic sex-chromosomes only. In addition, the formula of *Altica lythri* has also been found in eighteen other species of *Altica* mostly from North America (Smith and Virkki, 1978).

The karyotypic formulae of the three species of *Chaetocnema* studied differs slightly from the primitive one. *Ch. conducta* and *Ch. tibialis* with $2n = 22$, have probably derived from the ancestral karyotype by one centric fusion which possibly affected to the sex-chromosomes of the second species also as the $Xy_r$ system seems to indicate. Furthermore, in the case of *Ch. chlorophana* two centric fusions would be necessary to explain the origin of its $9 + Xy_p$ karyotype from the ancestral one.

All the remaining analyzed species have shown higher chromosome numbers than the primitive one and such increase could be accounted for by the effect of centric fissions. Among the four *Aphthona* checked two have $2n = 28$ and two more have $2n = 32$, the sex-bivalent seems to be chiasmatic but it is not clearly established yet.

The range of chromosomal variation in number found in the species of *Longitarsus* is similar to that of *Aphthona*. The three species chromosomally analyzed have $2n = 26$, $2n = 28$ and $2n = 30$. Unfortunately, nothing can be said about the sex-bivalent in the three examined species of *Longitarsus*. But the karyotype of *L. tabidus* has been worked out and is shown to be constituted by metacentric chromosomes belonging to three groups of sizes. The only *Longitarsus* previously analyzed was *L. oakleyi* Blake, from Puerto Rico, having a formula of $13 + X$ (Smith and Virkki, 1978) which differs from that of *L. tabidus*, $13 + Xy$, by the loss of the y-chromosome.

Three other species in our report possess high chromosome numbers: *Psylliodes chrysocephala* $2n = 36$, *Podagrica menetriesi* $2n = 40$, and *Sphaeroderma testaceum* $2n = 52$. To explain only the gross chromosomal changes involved in the origin of these three species from the primitive karyotype, we have to assume six, eight and fourteen centric fissions, respectively. On karyological grounds, all the three later genera are apparently more evolved than the former ones, and even the number of *S. testaceum* is the highest so far recorded in Alticinae.

The existence of the tribes Alticini, Chaetocnemini, Psylliodini, Podagricini and Sphaerodermini (Bechyné, 1968) is not contradicted by chromosomal data but the separation between Aphthonini and Longitarsini is not supported by their range of chromosome numbers, quite similar in the small sample of species examined of *Aphthona* and *Longitarsus* genera. Nevertheless, much more species of each tribe should be subjected to cyto-

genetic analysis to set up solid conclusions on phylogenetics and interrelationships between the different genera and tribes of Alticinae. Our first karyological contribution to the chromosomes of flea-beetles must be followed by further researches and not only at the level of **alpha** karyology but at much deeper levels of knowledge also. Besides that, a study of the number of spermatozoa per bundle in some representative species of European Alticinae would be extremely useful. When such projects will be developed we should have good perspectives of solving the main problemes posed by the systematics and evolution of Alticinae.

## References


Bechyné, J. 1968: Contribution à la faune du Congo (Brazaville). Mission A. Villiers el A. Descarpentries. – LXXXI. Coléoptères Alticidae. – Bull. Inst. fr. Afr. noire sèr. a, 30: 1687–1728

Dobzhansky, Th. 1951: Genetics and the origin of species. – 3rd. ed., Columbia Univ. Press, New York, 446 pp.

Hubby, J. L. and Lewontin, R. C. 1966: A molecular approach to the study of genic heterozygosity in natural populations. I: The number of alleles at different loci in *Drosophila pseudoobscura*. – Genetics **54**: 577–594

Lewontin, R. C. and Hubby, J. L. 1966: A molecular approach to the study of genic heterozygosity in natural populations. II: The amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. – Genetics **54**: 595–609

Mayr, E. 1963: Animal species and evolution. – The Belknap Press. Harvard Univ. Press, Cambridge, Mass., 787 pp.

Petitpierre, E. 1975: Notes on the chromosomes of ten species of the genus *Chrysolina* Mots. (Col. Chrysomelidae). – Genetica **45**: 349–354

— — 1976: Further cytotaxonomical and evolutionary studies on the genus *Timarcha* Latr. (Coleoptera: Chrysomelidae). – Genét. Ibér. **28**: 57–81

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

Smith, S. G. and Virkki, N. 1978: Coleoptera. – In Animal Cytogenetics 3, Insecta 5 (B. John Ed.). – G. Borntraeger, Berlin–Stuttgart, 366 pp.

Turner, B. J. 1974: Genetic divergence of Death Valley pupfish species: Biochemical versus morphological evidence. – Evolution **28**: 281–294

Virkki, N. 1969: Sperm bundle and phylogenesis. – Z. Zellf. **101**: 13–27

— — 1970: Sex chromosomes and karyotypes of the Alticidae (Coleoptera). – Hereditas **64**: 267–282

White, M. J. D. 1978: – Modes of speciation. – Freeman, San Francisco, 455 pp.



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