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The Cytogenetic System of Oedionychina (Alticinae)

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ABSTRACT. — The subtribe Oedionychina of the Neotropical flea beetles is distinguished by many unorthodox cytological features. The chromosome number is relatively conservative, the prevailing diploid and male meiotic numbers being 2n=22 and 10+X+Y, respectively. The karyotypes vary principally by the size and shape of the large sex chromosomes (about 50% of total karyotype length). This variation is notable also in the interior structure of the sex chromosomes, especially Y, where C-banding and Ag-banding mark species-specific intercalar bands. A secondary nucleolus-formation, which might be based on gene amplification, produces large quantities of a granular substance (ribosomal protein?) in the cytoplasm of spermatocyte I, apparently to the benefit of the embryo. Thus sperm cells are rendered very large (up to 4.5 mm long), and special rearrangements are needed in the testes for their assembly and transportation. Relevance of such features with genetics, mitosis theory, and systematics, are briefly discussed.

During the past 25 years, cytomorphological aspects of the Neotropical genera of the flea beetle subtribe Oedionychina have been studied (see Smith and Virkki, 1978, for references). Both chromosomal and extrachromosomal cytology show unorthodox features relevant to their genetic system. This paper briefly summarizes the results.

MATERIAL AND METHODS

Specimen material is comprised of Antillean as well as continental Neotropical Oedionychina. Meiosis in adults and embryonal mitoses, which were arrested with colchicine (Virkki, 1983), were the main cell sources. Most observations were made with a phase contrast microscope, on unstained squash preparations freshly made on albuminized slides. When albuminizing the slides, heating of the thin albumen sheath (Virkki, 1983) was always found essential for good squashes. Other, more complicated techniques, are given in the original papers, e.g., gamma radiation (Virkki, 1979), electron microscopy (Virkki, 1976; Virkki and Kimura, 1978), observation of living mitosis (Virkki, 1973a) and meiosis (Virkki, 1971, 1972), banding techniques (Virkki, 1983). Large size and fragility of the spermatocytes limit use of diverse tap-

ping and air drying techniques, although the latter technique, despite its wastefulness, has yielded good results (Smith and Virkki,1978, p. 139).

RESULTS AND DISCUSSION

I. The Chromosomes

The Number. The prevalent chromosome number is 2n=22, including the sex chromosomes X and Y. The latter comprise over 50% of the total karyotype length (Fig. 1). Some *Asphaera* ssp. have multiple sex chromosomes, up to 1X plus 7Y (Fig. 6). Changes in autosomal number are rare, but reduction to 16, and increase to 44 have been recorded (see the chromosome list in Smith and Virkki, 1978). Karyotypes are better distinguished by differences in arm relations and in sex chromosome shapes and sizes.

Neotropical karyotypes are conservative in this respect, while the rearrangements leading to their genesis might be more evident in the little studied Old World Oedionychina, as well as in the sister subtribe Disonychina that have more variable chromosome numbers and sex chromosome systems.

Radiosensitivity. Due to large target size, the sex chromosomes are frequently broken with even acute doses under 200r (Virkki, 1979). Rearrangements, including translocations, are profusely produced (Fig. 2). Still, naturally occurring rearrangements have never been recorded, not even in Pocos de Caldas, a site of high natural radioactivity in Brazil (Virkki, unpubl.).

Heterochromatin. By C-banding criteria, the large sex chromosomes are mainly composed of euchromatin. The Y chromosome often shows prominent intercalar blocks of constitutive heterochromatin. Small procentric blocks of it occur in most chromosomes (Figs. 3, 4).

About 50% of all genes are sex-linked, if the sex chromosomes are really euchromatic. They do not need to be so, because all coleopteran heterochromatin is not C-bandable (Smith and Virkki, 1978).

Pairing and Recombination. The autosomes pair normally in males, with a strictly distal distribution of the single chiasma. The sex chromosomes do not conjugate but form a distance bivalent or -multivalent (Figs. 5, 6). This takes place in a separate, peripheral spindle-portion ("sex spindle"), which can be dislocated by micromanipulation (Nicklas and Virkki, unpubl.) and often accidentally by squashing (Fig. 5). All chromosomes pair in females. The behavior of the XX bivalent suggests lack of chiasmata (Smith and Virkki, 1978, p. 138).

Such pairing and chiasma patterns render the genetic recombination very restricted, even though the autosomal chiasma distribution of the female is still unknown.

Division mechanics. Normally, the last points keeping the chromatids together in mitosis and Metaphase II (M II) of meiosis occur at both sides of the centromere. Such points have been called *collochores* (Virkki, in press). They are distally located in the autosomes of Oedionychina spermatogenesis, which creates a "V-look" (instead of "Λ-look") in A I autosomes, and a "bivalent look" of the autosomes in M II profiles (Figs. 4, 7). Sex chromosomes, and some autosomes of a similar condensation pattern as in the sex chromosomes (Fig. 5), have retained proximal collochores (Virkki, Memor. VI. Congr. Latinoamer. Genet., Maracaibo, Venezuela, 1983, in press).

Oeionychina share distal collochores with certain other Coleoptera, such as Carabidae (Serrano, 1981), Lampyridae (Smith and Virkki, 1978), Elateridae: Pyrophorini (Virkki et al., 1984), and Stylopidae (Ferreira et al., 1984).

Nucleolar organization. Two homologous autosomes assemble the nucleolus in mitotic interphases (Fig. 8). The sex chromosomes are not involved. Chains of droplet nucleoli are formed in the long-lasting diplotene of the male (Fig. 9). Each droplet developes a lateral granular component. After coalescence and notable disintegration, only one to two nucleoli remain, usually in the "sex spindle." They may persist until A I (Virkki, 1971).

Silver staining for nucleolus organizers marks species-specific "Ag-bands" in the Y chromosomes (Fig. 10). Silver stains also kinetochores (Fig. 11) and adjacent procentric regions, if they are condensed (Fig. 10, 12).

EM studies suggest that the main bulk of fibrillar material of the droplet nucleoli migrates through nuclear pores to the outer nuclear membrane, where it continues producing the granular material. This would mean amplification of rDNA. In *Alagoasa,* and related genera formerly under *Oedionychus,* nuclear pores of spermatocyte I are aggregated in "sieves" (NSC = nuclear sieve complex) where the fibrillar material functions (Virkki and Kimura, 1978). *Omophoita* still has the age-old random distribution of the pores.

II. Extrachromosomal Cytology

Spindle. Much has been written on the natural *vs* artefactual character of chromosome clumping just prior to the congression for M I. Observation of prophasic spermatocyte I of Oedionychina shows that the process is natural and occurs prior to the disintegration of the nuclear envelope (Virkki, 1976). Developing asters of prophase I push the entire nucleus against the opposite cell wall, where it becomes flattened, with a minimum of envelope rupture (Fig. 13). After one hour the nuclear envelope has disintegrated, and the autosomes congress for M I. The sex chromosomes congress at the clump site to form the distance bivalent and "sex spindle." Both A I and A II spindles are drum-shaped. At T I, a phragmoplast-like structure develops, as in plants,

but becomes substituted by a ring that strangles the equatorial plane in an animal fashion (Inoué and Virkki, unpubl.)

The sex chromosomes of an Oedionychina distince bivalent are *syntelically* oriented, i.e., both chromatidal centromeres of a chromosome are oriented towards the same pole. This distinguishes them from the univalent sex chromosomes of Alticini *(Altica, Macrohaltica, Lysathia, Hermaeophaga)* where each univalent is *amphitelically* oriented, i.e., the two chromatidal centromeres are oriented towards opposite poles.

No birefringence is seen between the distance-paired sex chromosomes (Inoué and Virkki, unpubl.). Their metaphasic movements are interdependent to some degree, probably due to a gelatinous substance which becomes more conspicuous in denuded spindle apparatuses (Virkki, 1971).

Spermatogonia and Sperm Cells per Bundle. Four simultaneous mitoses of definitive (encysted) spermatogonia produce 2^4=8 spermatogonia. The last mitosis is, however, an unequal "budding" (Fig. 14), resulting in four small, perishing buds and four large surviving spermatocytes (Virkki, 1973a). Such unequal divisions could be engaged in regulation of supernumerary chromosomes or other unwanted/wanted organelles (Smith and Virkki, 1978).

All definitive spermatogonia are fusomally interconnected; due to migration of fusomes (Smith and Virkki, 1978, p. 12) towards a common branching-point, a fusomal cytophore is formed. After two meiotic divisions, the number of spermatids is 16 per cyst, resulting in 16 very large (up to 4.5 mm) sperm cells per bundle (spz/b). This is the lowest known spz/b. Comparison with other flea beetles suggests that the reduction from the highest coleopteran spz/b=512 has occurred stepwise (by halves) and irreversibly during the evolution, and this tendency might exist also in other arthropods with sperm bundles (Virkki, 1969, 1970, 1973b). In other words, a corollary of Dollo's law concerning meristic characteristics might apply; that is, "If the number of spermatogonial mitoses per cyst (and thus spz/b) is reduced during the evolution, the reduction is irreversible."

Spermiogenesis and Sperm Transportation. Because sperm cells are nearly as long as the beetles themselves, the question arises how thousands of them are simultaneously produced in testis follicles only 0.4 mm across, and how are they transported out of the testis. This was studied by Tanya Bruck (1978).

The elongating sperm tails are deposited at the equator of the follicle. Bundle after bundle, this is repeated, until a donut-like deposit is formed. Then, the bundles coil out of the "donut" (Fig. 15); the coiling starts from the middle of each bundle. This is the only flagellar activity of these sperm cells.

Since the bundles do not get tangled, they can be easily transported to the female. The large size of the sperm coils and the small size of

spermatheca probably permit only a small portion of the transferred spermatozoa to be deposited in the spermatheca.

CONCLUSIONS AND RECOMMENDATIONS

The modern subtribe Oedionychina is cytologically still quite uniform in the Neotropics, although incipient signs of probable evolutive branching-off do exist, such as the tendency towards multiple sex chromosomes in *Asphaera* and presence of nuclear sieve complexes in genera derived from earlier *Oedionychus*.

Oedionychina is distinguished by an accumulation of unusual cytological features, and, as Bateson said (*in* White, 1973, p. 762), such exceptions are to be treasured by scientists.

Systematics would benefit from comparison of banded Oedionychina karyotypes, and especially from C- and Ag-banding of the large Y chromosomes. The significance of the spz/b determinations is still under doubt (Crowson, 1981), and further studies are needed. As Rosen-Runge (1977) mentions arrangements of envelopes surrounding germ cells might be good indicators of phylogeny and the microenvironment within the envelopes, quite isolated from the remainder of the body. Special conditions favoring "Dollo's corollary" might be present in the cysts.

Genetics of Oedionychina is still unknown, but offers a challenging field of study. Provided that the sex chromosomes are euchromatic and exempt from crossing over, they should evolve like apomictic karyotypes; by accumulation of gene and chromosomal mutations. Strictly terminal chiasmata place the autosomes in a similar position.

The large size of spermatocytes and of the unpaired sex chromosomes is well suited for experimental intervention in chromosomal orientation and segregation. The bipartite nature of the M I spindle invites experimentation on autosomes *vs* sex chromosomes by microinjections, for instance. Further, phragmoplast studies should elucidate the differences between plant and animal cytokinesis.

Why has selection favored the large, awkward sperm cells, for which special storage and transportation arrangements are needed in the male genital system? The answer apparently lies in the importance of the large amounts of granular substance produced in the diffuse diplotene. Probably it consists of ribosomal proteins for the benefit of the future embryo. The Y chromosome somehow controls this diplotenic activity. The sex chromosomes seem to be in a key position for studies of Oedionychina evolution and speciation. Experimental sex chromosome strains could be produced with low, vital radiation doses, for evaluation of the genetic significance of these large chromosomes.

The above-mentioned studies are waiting to be made, especially after some of the pioneering cytomorphological studies are completed. Interesting data for comparison with the standard *Drosophila* genetics would certainly emerge.

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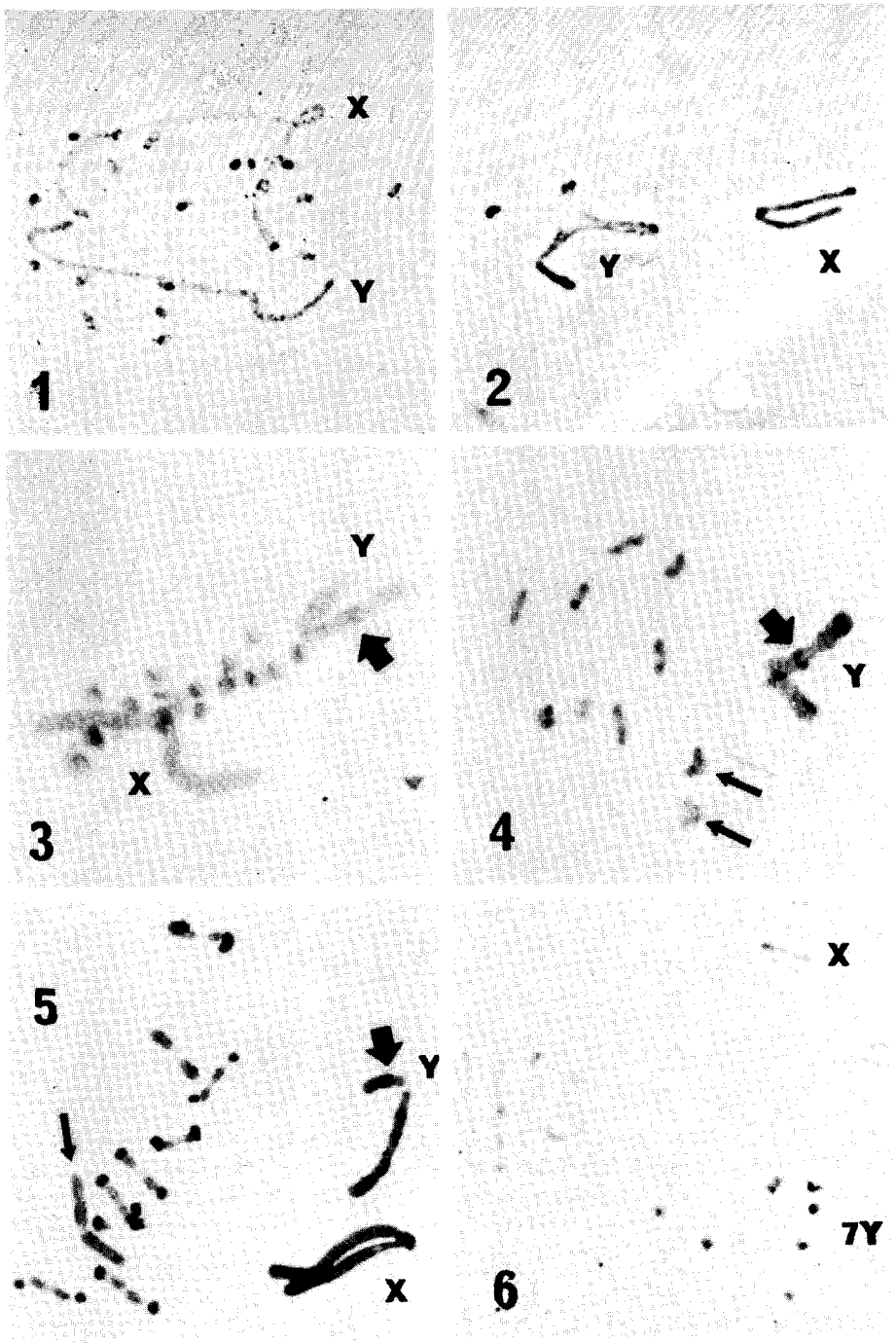
Figs. 1-6. Fig. **1,** *Alagoasa bicolor* (L.) (Puerto Rico). Mitosis of embryo. Twenty small autosomes and giant-size X and Y. Phase contrast. Fig. **2,** *Omophoita cyanipennis* F. (Puerto Rico). Sex chromosomes 84 h after an acute gamma does of 500r. X intact; Y-to-autosome translocation. Phase contrast. Fig. **3,** *Omophoita octoguttata* F. (Brazil). C-banded spermatogonial mitosis. Like the autosomes, X has only one procentric band; Y has several intercalary ones; arrow shows the strongest of them. Fig. **4,** *Omophoita octoguttata* F. (Brazil). C-banded M II with Y chromosome: arrow shows the strongest band of the latter. "Bivalent look" of the autosomes. The thin arrows show two chromosomes still in the process of opening-out from their anaphasic condition (see Fig. 7). Fig. **5,** *Omophoita personata* Ill. (Brazil). C-banded M I. Displacement of the sex chromosomes (and "sex spindle") due to squashing. Note that the strongest C-band of Y is in the short arm (arrow), not in the long one as in *O. octoguttata.* Autosome (thin arrow) of similar condensation pattern as in sex chromosomes shows proximal collochore in meiotic anaphases. Fig. **6,** *Asphaera* sp. (Brazil). M I. Nine autosomal bivalents (only a few visible), and 1X + 7Y. Phase contrast. (Magnifications: Figs. 1, 2 and 5: 1040×; Figs. 3 and 4: 1200×; Fig. 6: 1135×.)



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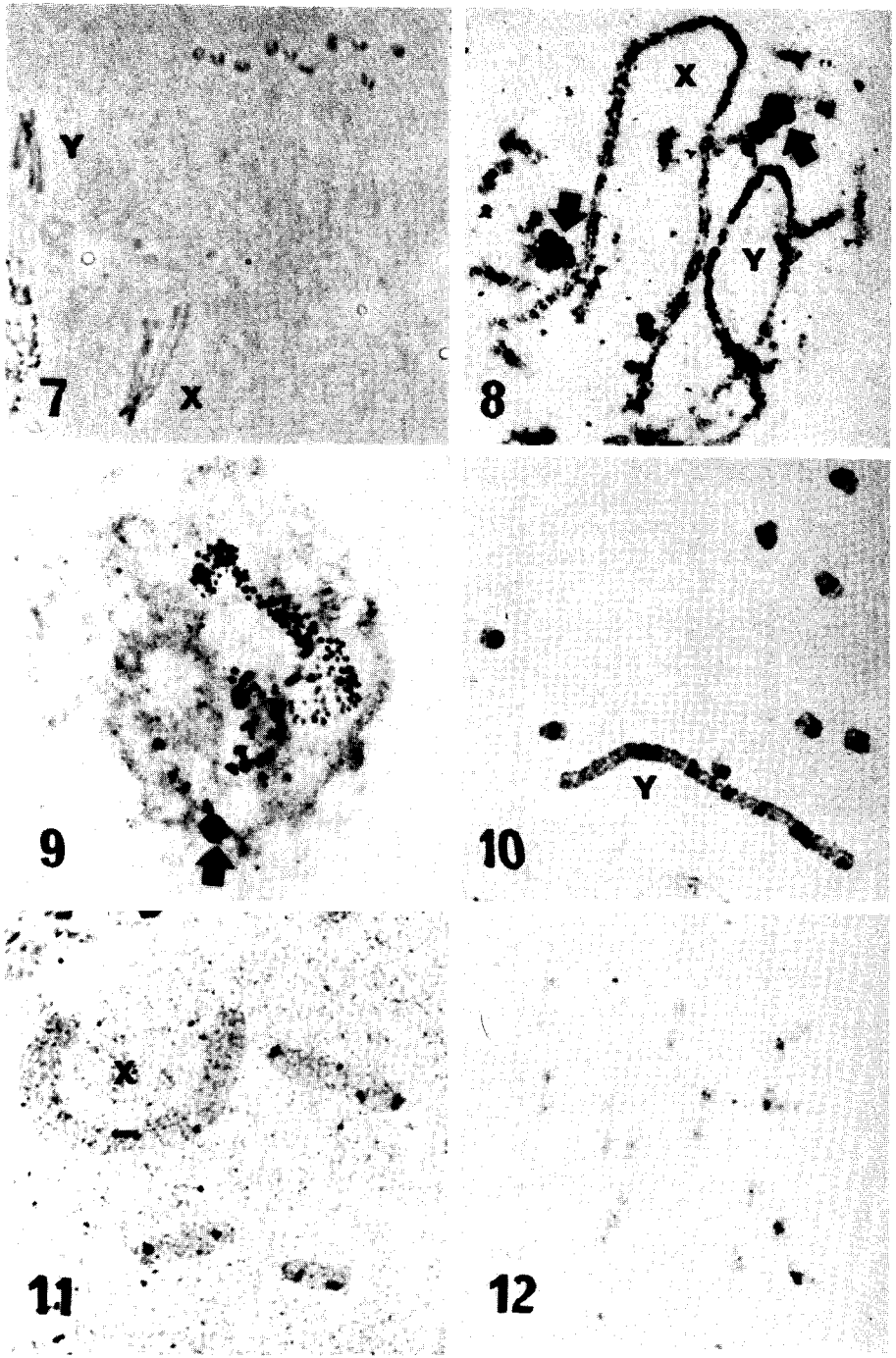

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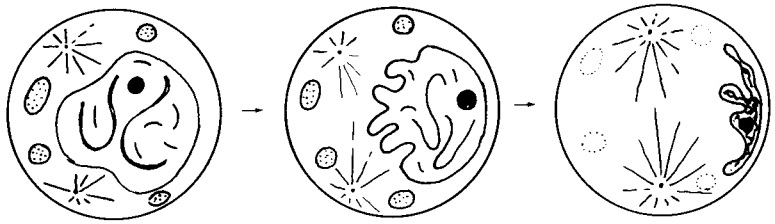
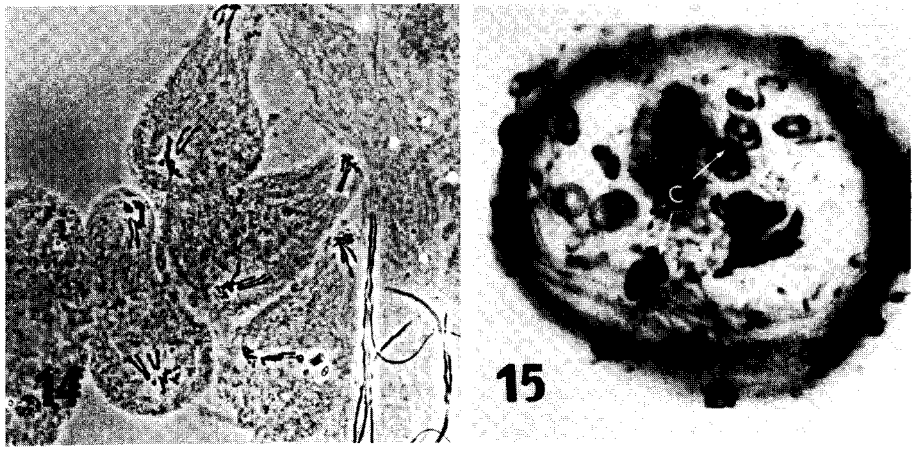


Fig. 13. Contraction ("clump") stage of spermatocyte I in Oedionychina. Asters push and flatten the nucleus against opposite cell wall. Nuclear envelope will disintegrate later (according to Virkki 1972, 1976).



Figs. 14 & 15. Fig. **14,** *Alagoasa rufina* Ill. (Perú). The last spermatogonial mitosis of Oedionychina is unequal, resulting in four large spermatocytes I per cyst, and four vanishing buds. Phase contrast (see Virkki, 1973a for observation of the same in living cells). Fig. **15,** *Alagoasa bicolor* (L.) (Puerto Rico). The donut-shaped deposit of sperm bundles excised from a testis follicle. C: sperm bundles coiling out of the "donut" (from Bruck, 1978). (Magnifications: Fig. 14: 250×; Fig. 15: 44×.)

Figs. 7-12. Fig. **7,** *Alagoasa bicolor* (L.) (Puerto Rico). A I. Sex chromosomes have proximal collochores that prevent separation of chromatids. All autosomes have distal collochores. Phase contrast. Fig. **8,** *Alagoasa blanda* (Har.) (Brazil). Silver-stained somatic prophase. Arrows show two nucleoli assembled at homologous NOR-chromosomes. As usual in somatic mitoses of Oedionychina, Y more condensed than X. Fig. **9,** *Alagoasa bicolor* (L.) (Puerto Rico). Diffuse diplotene in male. Original nucleolus (arrow) and a chain of secondary droplet nucleoli. Silver staining. Fig. **10,** *Omophoita* near *personata* Ill. (Brazil). Silver marks 5 major bands in Y, and condensed procentric chromatin in autosomes. Compare to similar autosomal structure in unstained (Fig. 1) and C-banded (Figs. 3 and 5) condition. Fig. **11,** *Paranaita bilimbata* Baly (Brazil). Silver marks the kinetochores. Note the unusual breadth (4 granules) and lateral position of X chromosome kinetochore. Fig. **12,** *Alagoasa januaria* Bech. (Brazil). M I, autosomes only. Silver has marked only the kinetochores in most bivalents. Two largest bivalents, formed by metacentrics, show additional procentric silver, due to condensed chromatin. (Magnifications: Fig. 7: 752×; Figs. 8 and 9: 1160×; Figs. 10 to 12: 1200×.)

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ABSTRACTS ONLY
(full text published elsewhere)

Genitalia, Taxonomy, & Distribution of Nearctic *Plateumaris*
(Coleoptera: Chrysomelidae)

ABSTRACT. — Donaciinae is a virtually cosmopolitan group, consisting of 5-9 genera (depending on authors). The genus *Plateumaris* is not well defined and its relationships to other Donaciinae are unclear. As presently conceived the genus includes 16 nearctic species (about 34 worldwide). Most nearctic species belong in the subgenus *Euplateumaris*. Distribution patterns show full-glacial displacement southwards, as evidenced by relictual southern higher-altitude populations. Populations isolated in eastern and western refugia during the Pleistocene have diverged slightly and complete introgression is prevented by a mid-continental grassland barrier. Eastern North America shows a relatively great degree of endemism, while only one variable species is limited to the western cordillera. Structure of the endophallus (internal sac) is mostly species-specific; it is also a useful character system for determining species-groups. Other genitalic characters are potentially useful at generic and subgeneric levels. Revisionary work on world species of *Plateumaris* and other genera is being done based on genitalia. Phylogenetic reconstruction cannot be accomplished without consideration of the world fauna. The nearctic fauna of *Plateumaris* is composed of several species-groups whose sister-taxa occur in other regions.

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Phaedon fulvescens: A possible control agent of *Rubus* in the Tropics

ABSTRACT. — In the Mascareignes Islands and Madagascar, two *Rubus* species have been introduced: *Rubus moluccanus* and *R. alceaefolius* or possibly a hybrid of both. The original species had been imported deliberately 200 years ago from the highlands of North Vietnam. Actually the *Rubus* are pests devasting pasture areas, namely in the Highlands of La Reunion.

After analysing the natural enemies of *Rubus* in the Highlands of Tam Dao (1500 m alt.) in North Vietnam, I selected several fungi, mostly rusts, and some Coleoptera, of which only few Chrysomelidae seem entirely selective, i.e., not attacking roses and strawberries as other rubiophagous beetles do.