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# Enzymatic and chronological discrimination in *Quadroppia quadricarinata* (MICHAEL, 1885) (Acari, Oribatida)

**Abstract**

The esterase contents of the *maritalis* and *virginalis* subspecies (identified by LIONS, 1977, 1982) of the worldwide distributed oribatid mite *Quadroppia quadricarinata* (MICHAEL, 1885) are different. The enzymatic polymorphism ist more pronounced within *maritalis* than *virginalis*; this may be referred to distinct adaptations to the environment. In other respects, the absence of vertition affecting the anterior solenidion of tarsus II within *virginalis* indicates the non-recent divergence of the two subspecies. Finally, our observations suggest a correspondence within the oribatid phylum between the numerical regression dealing of small organs and the reduction of enzymatic polymorphism.

**Résumé**

**Discrimination enzymatique et chronologique chez *Quadroppia quadricarinata* (MICHAEL, 1885) (Acari, Oribatida)**

Le contenu en estérases des sous-espèces *maritalis* et *virginalis* identifiées par LIONS (1977, 1982) chez l'Oribate cosmopolite *Quadroppia quadricarinata* (MICHAEL, 1885) est différent. Le polymorphisme enzymatique est plus important chez *maritalis* que chez *virginalis*; ceci correspond sans doute à des adaptations distinctes au milieu. Par ailleurs, l'absence de vertition touchant le solénidion antérieur du tarse II chez *virginalis* indique que la séparation des deux sous-espèces n'est pas récente. Nos observations suggèrent enfin une correspondance dans le phylum des Oribates entre la régression numérique des petits organes et la réduction du polymorphisme enzymatique.

**Kurzfassung**

**Enzymologische und chronologische Unterscheidung bei *Quadroppia quadricarinata* (MICHAEL, 1885) (Acari, Oribat.)**

Die beiden, von LIONS (1977, 1982) beschriebenen Unterarten *maritalis* und *virginalis* der weltweit verbreiteten Hornmilbe *Quadroppia quadricarinata* (MICHAEL, 1885) unterscheiden sich in ihrem Esterasen-Gehalt. Der enzymatische Polymorphismus ist bei *maritalis* stärker ausgeprägt als bei *virginalis*, was zweifelsohne mit bestimmten Anpassungen an den Lebensraum zusammenhängt. Andererseits deutet das Fehlen einer Vertition im Bereich des vorderen Solenidium auf Tarsus II bei *virginalis* an, daß die Trennung in die beiden Unterarten schon länger zurückliegt. Unsere Beobachtungen lassen einen Zusammenhang zwischen der numerischen Regression kleiner Organe und der Reduktion des enzymatischen Polymorphismus in der Gruppe Oribatida vermuten.

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## 1. Introduction

Just as in the majority of arthropods, the specific and subspecific discrimination between mites is essentially based on comparisons of morphological characters. Ecological or ethological data are very seldom taken into account in Systematics of mites. As far as taxonomic or phylogenetic applications of our biochemical knowledge are concerned, those may be considered as non-existent because the number of works devoted to the protein content analysis of mites is very limited.

OGITA & KASAI (1965) were the first to study the enzymes of Acari. BLANK (1979), SILBERSTEIN et al. (1979) and DUJARDIN et al. (1981) made an inventory of the esterases of different species of Actinedida and Acaridida. Several enzymes were examined and compared within Gamasida by CICOLANI et al. (1981). WARD et al. (1982) analysed the malate dehydrogenase of three species of spider mites. In the case of Oribatida (i. e. cryptostigmatic mites), only one study was made till now; it refers to the examination of esterases in *Platynothrus peltifer* (VERA & WAUTHY, 1983).

The purpose of the present article is, first of all, to establish if differences exist in the enzymatic contents of individuals belonging to the subspecies identified in the populations of the oribatid *Quadroppia quadricarinata* (MICHAEL, 1885) sensu lato.

The most recent and available information (BALOGH, 1983) shows that the genus *Quadroppia* comprises of six species. Amongst these species, *Quadroppia quadricarinata* is a cosmopolitan taxon, mentioned worldwide in practically all faunistic and ecological publications. It is usually present in soil organic horizons of forests as well as of meadows (LEBRUN, 1971). In western Europe, LIONS (1977, 1982) showed the existence of two subspecies within this taxon; they are indicated by *Q. quadricarinata maritalis* and *Q. quadricarinata virginalis.* The distinction between the subspecies (in other respects, they definitely are very similar from a morphological standpoint) is based on three essential points:

(1) the size of *marginalis* is appreciably larger than *maritalis* (for example, mean body length of virginalis is ~ 220 µ and mean body length of *maritalis* is ~ 195 µ);

(2) in the case of *maritalis,* the tarsus of leg II shows two solenidia $\omega$; within *virginalis,* however, only one solenidion $\omega$ is present (solenidia are hair-like organs inserted on the legs and palp of oribatid mites; they have been observed to have a canalicule over all their length and to

lack actinopilin; GRANDJEAN (1961) supposes they have a particular sensitive role);
(3) in the case of *virginalis* reproduction is supposed to be parthenogenetic insofar as collected individuals are always female; within *maritalis*, however, collected individuals belong to both sexes in an almost identical proportion.
From an ecological standpoint, the actually available data indicate little difference. Often enough the two subspecies are living together and *maritalis* usually is more abundant than *virginalis*. Moreover, their ecological preferenda are quite similar; nevertheless, one of us (WAUTHY, 1982) notes some discordance when preferenda referring to altitude or to certain characteristics of the vegetation that shelter them are concerned. Finally, in the light of the very few differences we have so far observed, the subspecies constitue a suitable subject for being compared by means of electrophoresis.
In an evolutionary context, giving differences between the two subspecies leads us to ask if their divergence is ancient or recent in the phylogenetic time. This aspect, the chronological one, constitutes the second part of this article.

## 2. Materials and Methods

Electrophoresis
All the individuals we used for electrophoresis originated from a forest stand; this stand, located in Marche-les-Dames (Belgium) constitutes a reference station for the laboratory of Ecology of the University of Louvain (station n. 5 of WAUTHY & LEBRUN, 1980). The site where the stand is located belongs to the ecological system of the Sambre-Meuse Trench. Its vegetation is an oak-beech forest and is quite typical of forest communities which are set up on sloping calcareous outcrops. All the individuals we studied were adults living in bryophytes which develop upon the soil of the station. Fragments of bryophytes were collected at three different periods (November 1982, February and May 1983) and put on Berlese funnels. Immatures were not investigated; first, because they are exceptionally extracted by Berlese funnels (some individuals were collected; but we could not link them for sure to adults), and second, because till now attempts to breed *Quadroppia quadricarinata* haven't been successful.
Sorting of individuals was effected using a microscope (enlargement: 400 x) and counting the number of solenidia $\omega$ on tarsus II. This determination is only possible if the individuals are immobilized during all the observation time. The method we used consists first, in putting individuals in lots of three or four on a microscopic slide, and second, in anaesthetizing them with diethylic ether up to the moment when they are spreading their legs again. The inventoried enzymes are esterases (EC 3.1.1.) and malate dehydrogenase (EC 1.1.1.37). We used a 1.5 % agarose gel as support. The gel buffer was 0.05 M phosphate (pH 7.0). Its dimensions were: 80 mm x 80 mm x 1.5 mm. Esterases were rendered visible by immersing gel for 20 minutes in a 0.1 M phosphate buffer solution (pH 7.0) containing 12.5 ml of substrate (0.5 gr $\alpha$-naphtylacetate and 0.5 gr $\beta$-naphtylacetate in 75 ml acetone, adjusted to 100 ml) at room temperature. The gel was stained for 20 minutes with 0.5 gr Fast Blue B powder. As it is not possible to reveal esterases using single individuals, we decided after different tests to electrophorese lots of 60 mites. After sorting the individuals, they were congelated, and then crushed with a 0.2 ml microhomogenizer (Bees-Knees tissue grinder, WHEATON, Milville, N. J., USA) in a drop of 1 % Triton x-100 solution. The homogenized extract was sucked up with a capillary tube and put in the slits drawn upon the support.

Chronological approach
This approach is essentially based on the evolutionary theories which involve small characteristics of mites. These theories were stressed by GRANDJEAN in a lot of papers (in order to reduce the bibliography contents, we refer the reader to GRANDJEANs paper of 1951). In the first instance, GRANDJEAN's theories try to explain the disappearance of small organs during phylogenetic time (i. e. the time that clocks measure; but there is a second type of time, independant of the first one, i. e. the ontogenetic time during which an individual develops from fertilized or mature egg to adult).
In the present case, only solenidia $\omega$ of tarsus II will be considered. Nevertheless, let us point out that for many authors (see for example, LOKKI, 1976, CUELLAR, 1977, WHITE, 1978), sexual reproduction would be considered as more „primitive" than the parthenogenetic one. Thus, from this standpoint, the distinction between the *maritalis* and *virginalis* subspecies in phylogenetic time is indubitable.

## 3. Enzymatic discrimination

In the case of malate dehydrogenase, no one zymogramme could be interpreted with certainty; the morphs rendered by the coloration remained too pale. Where esterases are concerned, an identical zymogramme is brought out at each of the three sampled periods. In the case of *maritalis*, the zymogrammes (fig. 1) show three active zones located on the anodal side; in the case of *virginalis*, only one zone of activity is revealed, this zone is also on the anodal side. The first active zone of *maritalis* appears not far away from the departure slit; the next two zones are located beyond the position of the sole active region of *virginalis*. Consequently, no one esterase of *maritalis* can be connected to the one of *virginalis*. Where *virginalis* is concerned, however, it is possible that several different isoenzymes are involved in the sole registered zone of activity. The technique we use is perhaps the cause. Indeed, in an agarose gel, proteins separate in accordance with their electric charges; two proteins with approximately the same total charge, but whose structure is very different, exhibit an identical migration rate (FERGUSON, 1980; ROLLINSON, 1980; WRIGHT, 1980). In this case, the active zone on the track of *virginalis* is twice as large as each zone of *maritalis*. Consequently, we can admit that at least two esterases are present within *virginalis*, these esterases are supposed to have different structure but quite identical charges. This hypothesis will, of course, be investigated in the future. However that may be, the *maritalis* individuals exhibit a larger enzymatic heterogeneity than the *virginalis* ones.
This observation leads us to investigate how esterases (as evidenced in the case of *maritalis*) are genetically controlled. Let us point out that the method we use does

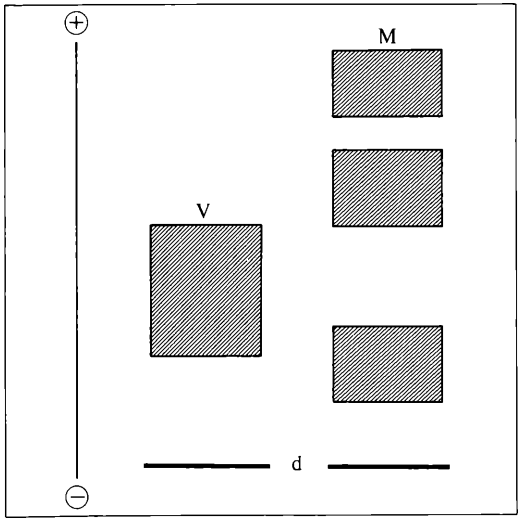


Figure 1. Esterase zymogrammes in *Quadroppia quadricarinata*. – V, *virginalis* subspecies; M, *maritalis* subspecies; d, departure slit; +, anode; –, cathode.

not allow us to analyse the esterase contents of one single individual; moreover, the exact number of loci coding for the concerned esterases is at the moment unknown. Therefore, three possibilities have to be considered:

(1) All the individuals are genetically identical; each individual is in possession of three different enzymes referring to the three registered zones of activity.

(2) There are three types of individuals whose abundances are approximately equal within the muscicolous population in Marche-les-Dames; each type possesses one or more different enzymes.

(3) We observe a dimeric protein, and in the present case, each polypeptide chain would be coded by a particular allele. So, the population we inventoried would be formed, on the one hand, by heterozygote individuals controlling the dimeric hybrid we observe in the centre of the track, and, on the other hand, by homozygote individuals responsible for the active regions located at the uttermost end of the track.

With regard to *virginalis*, the enzymatic homogeneity we observe proves indubitably that all the individuals are genetically identical. Moreover, we can be certain that other observations made on a greater number of populations than the one we consider at the present time would reveal an important genetic variability, as many authors indicate in the case of parthenogenetic species (Sokal, 1952; Suomalien & Suara, 1973; Ochman et al., 1980; Slobodchikoff, 1983). Therefore, we may presume that the population of Marche-les Dames has been set up from homozygous females which gained, in the meanwhile, the ability to reproduce by parthenogenesis.

In other respects, the differences we observe between the two subspecies correspond doubtlessly to dissimilarities in their adaptation to the environment. Gillespie & Kojima (1968) subdivided enzymes into two broad groupings, according to whether they have a role in glucose metabolism or not. As a matter of fact, the enzymes which are involved in glucose metabolism are less polymorphic than the others, i. e. their isoenzymes are not very frequent (let us point out that such an assessment can be established in practically all the phyla). Moreover, a weak isoenzyme diversity is found in the case of enzymes which act on a single substrate whose concentration remains constant (such enzymes intervene in main metabolic paths). On the other hand, an important polymorphism is observed in the case of enzymes whose substrates are also variable (such enzymes intervene in metabolic flow regulation, and thus, their polymorphism could be explained by the substrate diversity of the environment). These enzymes are the „variable substrate" ones of Kojima et al. (1970), and esterases are a part of them.

Many authors attribute to esterases a considerable isoenzymatic variability (Wilkinson, 1969; Ayala et al., 1972; Master & Holmes, 1975; Ramshaw & Eanes, 1978; Florence et al., 1982; Nakai, 1982; Adak et al., 1983; Hadacova et al., 1983; Schmidt-Stohn & Wehling, 1983). In pursuance of this isoenzymatic polymorphism dissimilarity, we could admit that:

(1) from a metabolic standpoint, the *maritalis* individuals are able to use a great number of substrates;

(2) from an ecological standpoint, the nutritional and energy resources of *maritalis* are more diversified than the ones of *virginalis*. In other words, diverging in phylogenetic time allowed *maritalis* and *virginalis* to occupy separate ecological niches; nevertheless, the niche breadth of *maritalis* is larger than the one of *virginalis*, but, in contrast, the niche of the latter is more specialised.

## 4. Chronological discrimination or test of evolutionary interpretation

Our aim is not to date precisely the moment of divergence of the two subspecies; we cannot know the past. In simple terms, we attempt to determine whether that divergence is an ancient or a recent one. If it were recent, there must still be some traces of it. Let us try to find these traces.

Therefore, we will keep the problem of phylogenic divergence of the two subspecies in a precise microevolutionary context and pose the question in a different manner. Let us consider the solenidia $\omega$ of the second leg, and suggest two hypotheses on the manner in which they evolved; these hypotheses are based on our present knowledge on solenidiotaxy of oribatid mites (Grandjean, 1964).

Let us suppose that the normal evolution of solenidia $\omega$

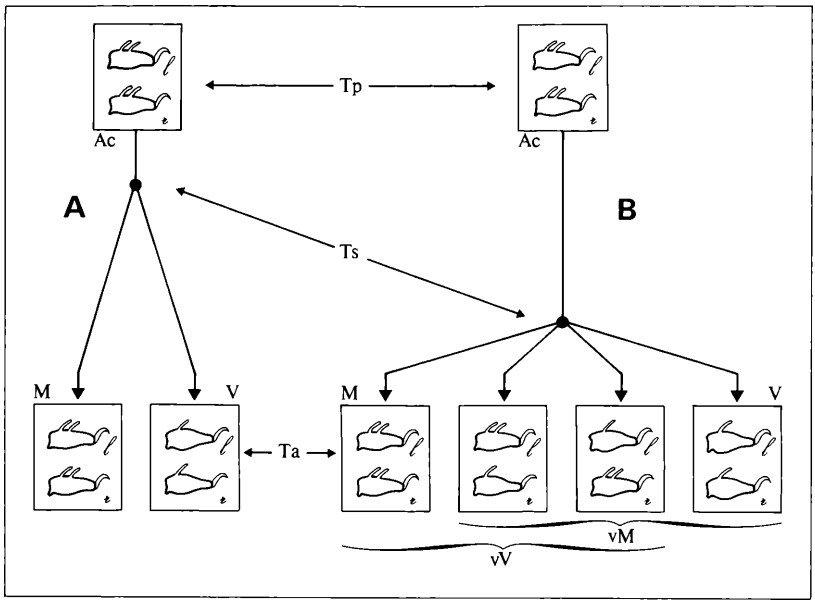


Figure 2. Diagrams showing the numerical evolution of solenidia of tarsus II within *Quadroppia quadricarinata maritalis* (M) and *Q. Q. virginalis* (V) subspecies, from a common hypothetical ancestor (Ac) living in time Tp. According to possibility A, the divergence of the subspecies (in time Ts) came true long before the present time (Ta). Following B, the divergence is later. – l, left tarsus II; r, right tarsus II; vM, possible vertitional states within *maritalis*; vV, id. within *virginalis*.

of tarsus II whithin the *Quadroppia quadricarinata* taxon manifests itself by numerical regression. The "primitive" state would be that of *maritalis* showing two solenidia $\omega$ and the "secondary" one would be the case of *virginalis* with only one solenidion (see annotation at the end). Let us also admit that the disappearance of the solenidion in *virginalis* would have occured by vertition, i. e. in a fundamentally unilateral way (by definition, vertition is a variation in the presence or absence of an idionymous organ observed in individuals of the same species and at the same ontogenetic level, provided that this variation is fundamentally unilateral and has an evolutionary significance; cf. GRANDJEAN, 1972). In this case, the phenomenon did not take place simultaneously for the left and right leg (i. e. in the course of phylogenetic time, the frequency of appearance of the concerned solenidion lessened within *virginalis*, and this frequency was not the same on the two sides of the individuals).

In the light of these hypotheses, the answer to the question asked will be to establish whether the presence or absence of the anterior solenidion in both subspecies is stable or not. If it is stable, the divergence of the two subspecies can be considered as an ancient one. Consequently, as seen in figure 2A, the great majority of the *maritalis* individuals within the present populations has two solenidia; by contrast, more than only one solenidion is not observed in the *virginalis* individuals (and vertitions are practically inexistent). If the divergence had occured recently (fig. 2B), the anterior solenidion would be sometimes present and sometimes absent in individuals belonging to both subspecies, and as likely on the right leg as on the left leg. Two remarks are inferred:

(1) the proportion of individuals showing vertition can not be expected to be identical in all existing populations; on the contrary, we can expect, as TRAVÉ (1973) indicates, a variable distribution of individuals concerned, being scattered in certain populations, concentrated in the others;

(2) bringing to light a higher frequency of vertition in *maritalis* populations has a different significance; in this case, the value of the anterior solenidion as a characteristic differentiating the subspecies is doubtful.

Finally, a study on the presence of absence or the anterior solenidion in many individuals, sampled from populations controlled by different varying ecological conditions, could solve the problem. The available information is:

(1) On 130 *virginalis* individuals originating in variable quantities from seven populations of the Provence region and one population of the Paris region, LIONS (1982) notes the presence of one single solenidion on all the legs II; this is the same in Marche-les-Dames where the observations were conducted on 55 individuals collected in September 1983.

(2) Within *maritalis*, LIONS (loc. cit.) shows three absences of left anterior solenidion (2 ♀ and 1 ♂) and one absence of both solenidia in the right leg II (1 ♂) on 289 individuals originating from 23 stands; in Marche-les-Dames, on 145 individuals two absences in the right (1 ♀ and 1 ♂) and one absence in the left (1 ♂) have been recorded.

From these observations, though fragmentary, we may infer that the disappearance of the anterior solenidion of tarsus II appears definitely to be an acquired characteristic by *virginalis*. On the other hand, the individual absences of the concerned organ within *maritalis* seem to be the forerunner of a regression, i. e. they an-

nounce its complete disappearance in the more or less long term. Nevertheless, the possibility that the observed missing solenidia are anomalies can not be denied (these are like vertitions, fundamentally unilateral in mites, GRANDJEAN, 1972); moreover, LIONS (1982) reveals several anomalies within *maritalis*, and certainly much more important in number in this subspecies than in *virginalis*. The study on the disappearance frequency of the anterior solenidion in other populations than those listed until now will allow to confirm this possibility.

**Annotation**

The presence of two solenidia $\omega$ II allows us to classify *maritalis* in category 2 C, as defined by GRANDJEAN (1964). The remaining solenidion of *virginalis* is probably homologous to the posterior solenidion ($\omega$1) of *maritalis*; its considerably backwards and a little paraxial position on the article seems to confirm it (see fig. 4 A and 4 B of LIONS, 1977). From a phylogenetic standpoint, we admit that the primitive state of the solenidion $\omega$ 2 is its presence; in that case, *maritalis* is still, but partially, in the primitive state; the secondary state is the absence of the solenidion $\omega$ 2 as in *virginalis*. It may be that the absence of $\omega$ 2 is the primitive state; if so, *virginalis* is still in this condition and, where *maritalis* is concerned, the evolution of the solenidion $\omega$ 2 would be first, its appearance in times past, and second, its disappearance by vertition at the present time. Such an evolution is maybe possible in oribatid mites, but anyhow it is very exceptional (GRANDJEAN, 1954). The other possibility (i. e. the remaining solenidion of *virginalis* is $\omega$ 2) could not be ignored because GRANDJEAN (1964) suggests that solenidia are able to displace a lot on the segments on which they are located. This means that the solenidion $\omega$1 has disappeared in *virginalis*; because the solenidion $\omega$ 1 II is larval in oribatid mites whose development we know, this possibility is in opposition to the rule of ontogenetic priority. Nevertheless, a doubt exists about the ontogenetic level in which the two solenidia appear.

On the other hand, other tarsal characters (and particularly the presence of proral setae on legs II, III and IV) distinguish very clearly *Quadroppia quadricarinata* from Oppiidae (GRANDJEAN, 1953). Later study of the immature instars would indicate whether to classify *Q. quadricarinata* with its subspecies as a new family or not.

## 5. Conclusions

The answers to the questions we asked are as follows:

(1) There exists a certain difference within the esterase contents of the *maritalis* and *virginalis* subspecies of the oribatid mite *Quadroppia quadricarinata*; moreover, the enzymatic polymorphism is less important within *virginalis* than *maritalis*.

(2) The disappearance of the anterior solenidion of tarsus II is apparently constant in *virginalis*; therefore, it is practically certain that the divergence of the two subspecies is not recent. This disappearance, as well as parthenogenesis indicates that *virginalis* is phylogenitically less „primitive" than *maritalis*, i. e. *virginalis* can be referred to a more youthful state in phylogenetic time (according to GRANDJEAN, 1954) than the latter subspecies. From a physiological standpoint, relationships between the diminution of the enzymatic variability and the reduction of the number of solenidia $\omega$ II observed in *virginalis* do not appear to be evident; but, from a phyletic standpoint, the full significance of this conclusion seems to be very important. Indeed, as many studies of GRANDJEAN confirm, there has been a regressive evolution (numerical) of several organs within the oribatid phylum. And, this regression concerns not only the solenidia of legs as studied herein, but also the simple rows of setae belonging to the appendages, the setae of the notogaster, the genital setae, the anal setae and so on. Consequently, could it be supposed that in the phylum of oribatid mites, the numerical regression of organs keeps pace with a numerical regression of enzymatic variability? Answering this question implies comparing the enzymatic contents of several species at each ontogenetic level, from each of six major oribatid phyletic groupings.

Up to the present however, two observations made by VERA (1984) on *Platynothrus peltifer,* i. e. a species pertaining to the Nothrina (this group is more primitive in phylogeny than the one to which *Quadroppia quadricarinata* belongs, as pointed out by GRANDJEAN, 1953) are positive proof of our hypothesis: (1) at the adult level, *P. peltifer* shows an important esterase variability much more important than that we observe in *Q. quadricarinata maritalis;* (2) this variability changes in the course of ontogeny; the protonymphs and tritonymphs show an esterase contents less diversified than that of the adults. Such variations of enzymatic polymorphism during ontogenetic time are not surprising at all. On the contrary, we actually know that in mites, there are independant relations between phylogenies and ontogenies, i. e. each ontogenetic level demonstrates an evolution peculiar to itself (GRANDJEAN, 1954); and, this definitely implies a peculiar specialisation regarding the exploitation of the milieu, the nutritional resources, etc.

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## 6. Literature

ADAK, T., SUBBARAO, S. K. & SHARMA, V. P (1983): Male specific esterases in certain Anopheline mosquitoes. – Mosq. News, **43**: 14–16; Fresno.

AYALA, F. J., JEFFREY, J., POWELL, R., TRACEY, M. L., MOURÃO, C. A. & PEREZ SALAS, S. (1972): Enzyme variability in the *Drosophila willistoni* group. 4. Genic variation in natural populations of *Drosophila willistoni*. – Genetics, **70**: 113–139; Austin.

BALOGH, J. (1983): A partial revision of the Oppiidae Grandjean, 1954 (Acari: Oribatei). – Acta Zool. Acad. Sci. Hung., **29**: 1–79; Budapest.

BLANK, R. H. (1979): Studies on the non-specific esterase and acetylcholinesterase isozymes electrophoretically separated from the mites *Sancassania berlesi* and *Tetranychus urticae*. – N. Z. J. Agric. Res., **22**: 497–506; Wellington.

CICOLANI, B., BULLINI, A. P. B. & BULLINI, L. (1981): Morphological and genetic differentiation between *Macrocheles glaber* and *Macrocheles perglaber*. – Int. J. Acarol., **7**: 221–224; Oak Park.

CUELLAR, O. (1977): Animal parthenogenesis. – Science (Wash. DC), **197**: 837–843; Washington.

DUJARDIN, J. P., FAIN, A. & DE DEKEN, B. (1981): Une microméthode d'électrophorèse en plaque d'amidon applicable à l'étude d'individus isolés de microarthropodes. – Ann. Soc. Belge Méd. Trop., **61**: 467–475; Brussels.

FERGUSON, A. (1980): Biochemical Systematics and Evolution. – 194 p.; Glasgow (Blackie and Son Ltd.).

FLORENCE, Z. L., JOHNSON, P. C. & COSTER, J. E. (1982): Behavioral and genetic diversity during dispersal: Analysis of a polymorphic esterase locus in southern pine beetle, *Dendroctonus frontalis*. – Environ. Entomol., **11**: 1014–1018; College Park.

GILLESPIE, J. M. & KOJIMA, K. (1968): The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. – Proc. Nat. Acad. Sci. U.S.A., **61**: 582–585; Washington.

GRANDJEAN, F. (1951): Les relations chronologiques entre ontogénèses et phylogénèses d'après les petits caractères discontinus des Acariens. – Bull. Biol. Fr. Belg., **85**: 269–292; Paris.

GRANDJEAN, F. (1953): Essai de classification des Oribates (Acariens). – Bull. Soc. Zool. Fr., **79**: 421–446; Paris.

GRANDJEAN, F. (1954): Les deux sortes de temps et l'évolution. – Bull. Biol. Fr. Belg., **88**: 413–434; Paris.

GRANDJEAN, F. (1961): Nouvelles observations sur les Oribates (1ere partie). – Acarologia, **3**: 206–231; Paris.

GRANDJEAN, F. (1964): La solénidiotaxie des Oribates. – Acarologia, **6**: 529–556; Paris.

GRANDJEAN, F. (1972): Caractères anormaux et vertitionnels rencontrés dans des clones de *Platynothrus peltifer* (Koch). – Acarologia, **14**: 454–478; Paris.

HADAČOVÁ, V., ŠVACHULOVÁ, J., KLOZOVÁ, E., HADAČ, E. & PITTEROVÁ, K. (1983): Use of esterase isoenzymes revealed by gel isoelectric focusing as an aid in chemotaxonomical study of the genus *Allium*. – Biol. Plant. (Prague), **25**: 36–42; Prague.

KOJIMA, K. I., GILLESPIE, J. H. & TOBARI, Y. N. (1970): A profile of *Drosophila* species enzymes essayed by electrophoresis. 1. Number of alleles, heterozygosities and linkage desequilibrium in glucose-metabolizing systems and some other enzymes. – Biochem. Genet., **4**: 627–637; New York.

LEBRUN, PH. (1971): Ecologie et Biocénotique de quelques peuplements d'Arthropodes édaphiques. – Mém. Inst. Roy. Sc. Nat. Belg., **165**: 1–204; Brussels.

LIONS, J. C. (1977): Au sujet de la chétotaxie des pattes et de la présence des poils proraux chez des Oribates proches du *Quadroppia quadricarinata* (Michael, 1885). – Acarologia, **19**: 540–551; Paris.

LIONS, J. C. (1982): Statistique sexuelle chez deux formes d'Oribates proches de *Quadroppia quadricarinata* (Michael, 1885). – Acarologia, **23**: 373–389; Paris.

LOKKI, J. (1976): Genetic polymorphism and evolution in parthenogenetic animals. 7. The amount of heterozygosity in diploid populations. – Hereditas, **83**: 57–64; Landskrona.

MASTERS, C. J. & HOLMES, R. S. (1975): Haemoglobin, isoenzymes and tissue differentiation. – 308 p.; Amsterdam (North Holland Publishing Company).

NAKAI, Y (1982): D genome donors for *Aegilops crassa* ($DDm^{Cr}M^{Cr}$, $DDD^2M^{Cr}M^{Cr}$) and *Ae. vavilovii* ($DDM^{Cr}S^PS^P$) deduce from esterase analysis by isoelectric focusing. – Jpn J. Genet., **57**: 349–360; Miskima.

OCHMAN, H., STILLE, B., NIKLASSON, M., SELANDER, B. K. & TEMPLETON, A. (1980): Evolution of clonal diversity in the parthenogenetic fly *Lonchoptera dubia*. – Evolution, **34**: 539–547; Lawrence.

OGITA, Z. & KASAI, T. (1965): A microtechnique for enzyme separation of individual spider mites with thin layer electrophoresis. – SABCO J., **1**: 117–120; Osaka.

RAMSHAW, J. A. M. & EANES, W. F. (1978): Study of the charge-state model for electrophoretic variation using isoelectric focusing of esterase-5 from *Drosophila pseudoobscura*. – Nature (Lond.), **275**: 68–70; London.

ROLLINSON, D. (1980): Enzymes as a taxonomic tool: a zoologist's view. – In: BISBY, F. A., VAUGHAN, J. G. & WRIGHT, C. A. (Edit.): Chemosystematics: Principles and practice: 123–146; Academic Press/London.

SCHMIDT-STOHN, G. & WEHLING, P. (1983): Genetic control of esterase isoenzymes in Rye (*Secale cereale* L.). – Theor. Appl. Genet., **64**: 109–115; Heidelberg.

SILBERSTEIN, A. J., FAIN, A. & HERIN, A. (1979): Esterase isozyme patterns of some astigmatic mites. – Int. J. Acarol., **5**: 1–14; Oak Park.

SLOBODCHIKOFF, C. N. (1983): Why asexual reproduction ?: Variation in populations of the parthenogenetic wasp, *Venturia canescens* (Hymenoptera: Ichneumonidae). – Ann. Entomol. Soc. Am., **76**: 23–29; College Park.

SOKAL, R. R. (1952): Variation in a local population of *Pemphigus*. – Evolution, **6**: 296–315; Lawrence.

SUOMALAINEN, E. & SAURA, A. (1973): Genetic polymorphism and evolution in parthenogenetic animals. 1. Polyploid Curculionidae. – Genetics, **74**: 489–508; Austin.

TRAVÉ, J. (1973): Les variations chaetotaxiques dans quelques populations de *Mucronothrus nasalis* Willm. (Oribate). – Acarologia, **15**: 521–533; Paris.

VERA, H. (1984): Différenciation de deux populations de *Platynothrus peltifer* à partir d'observations écologiques, biochimiques et physiologiques. – 237 p.; Ottignies-Louvain-la-Neuve (Ph. D. Thesis, University of Louvain).

VERA, H. & WAUTHY, G. (1983): Methodology for a definition of soil oribatid mite populations. – In: LEBRUN, PH., ANDRÉ, H. M., DE MEDTS, A., GRÉGOIRE-WIBO, C. & WAUTHY, G. (Edit.): New Trends in Soil Biology: 685–687; Dieu-Brichart/Ottignies-Louvain-la-Neuve.

WARD, P. S., BOUSSY, I. A. & SWINCER, D. E. (1982): Electrophoretic detection of enzyme polymorphism and differentiation in three species of spider mites (*Tetranychus*) (Acari: Tetranychidae). – Ann. Entomol. Soc. Am., **75**: 595–598; College Park.

WAUTHY, G. (1982): Synecology of forest soil oribatid mites of Belgium (Acari, Oribatida). 3. Ecological groups. – Acta Oecol. Oecol. Gen., **3**: 469–494; Montreuil.

WAUTHY, G. & LEBRUN, PH. (1980): Synecology of forest soil oribatid mites of Belgium. 1. The zoosociological classes. – In: DINDAL, D. L. (Edit.): Soil Biology as related to land use practices: 795–805; E.P.A. (560/12-80-038)/Washington.

WHITE, M. J. D. (1978): Modes of speciation. – 455 p.; San Francisco (Freeman and Co.).

WILKINSON, J. H. (1969): Isoenzymes. – 369 p.; London (Chapman and Hall).

WRIGHT, C. A. (1980): Chemosystematics: Perspective, Problems and Prospects for the Zoologist. – In: BISBY, F. A., VAUGHAN, J. G. & WRIGHT, C. A. (Edit.): Chemosystematics: Principles and practice: 29–38; Academic Press/London.