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ECOLOGICAL AND EVOLUTIVE ASPECTS

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G. Besançon, J.L. Bozza and G. Second (*)

Genome A, which determines the sativa complex of Orizae, is represented in Africa by the species O. longistaminata, O. breviligulata (now called O. barthii), O. glaberrima and O. sativa, which was probably introduced more recently. (1).

In this complex, O. longistaminata is a perennial allogamous, rhizomatous species. The others are annual autogamous species.

Figure 1 shows the distribution of the main endemic species of Oryza in Africa and Madagascar (CHANG, 1975).

The following points will be treated :

1 - Observation of the existing populations.
2 - Study of reproductive barriers.
3 - Analysis of enzyme variability.
4 - Discussion and hypotheses.

(1) For easier reading, the terms O. longistaminata and O. breviligulata are used in this paper, even though they do not form part of a coherent system.

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1 - Observation of the existing populations.

O. longistaminata is by far the most widespread of all the Oryza species on the African continent (fig. 1).

In West Africa, this species has frequently been found by the authors in Senegal, Gambia, Mali, Upper Volta and Ivory Coast, that is to say from the edge of the desert in the north (areas flooded by the Niger at Timbuktu) down to the coastal areas in the south. It does not however occur in the Basse Côte forest areas, although it is found around water holes in the coastal savanna alongside the ocean in Ivory Coast (GUILLAUMET and ADJANOHOUN, 1971). In this coastal savanna, the degree of light is high compared with the neighbouring forest areas and the available light intensity possibly acts as a determining factor in the distribution of the species.

Just like its area of distribution, the ecological sites of O. longistaminata are the most varied of the Oryza species in Africa. Samples of populations have in fact been taken in the salt lagoons in the Casamance Delta as well as in the flooded areas (up to 4 m of water) in the inland Niger delta in Mali, and also in dry, sandy rice fields in Senegal. It is found in both running and stagnant water, in rivers that dry up during the dry season and almost permanent ponds.

In comparison, the annual form, O. breviligulata, has much more specific ecological requirements. It is adapted to rivers in the savanna areas. This species seems to be found specifically in rivers that are stirred up by cattle coming to drink. The fact that the soil is enriched in organic and mineral elements and that seeds are trodden into the soil may be thought to play an important part in the evolution and dissemination of the species whose characteristic features are high production of large seeds and very heavily aristate spikelets. A weed form of O. breviligulata, which invades cultivated rice, is also found.

In the rivers disturbed by cattle, where O. longistaminata and O. breviligulata are sympatric, the two species seem to share the ground; O. longistaminata grows in the deeper parts or is found as isolated plants. Similarly, in the Niger Delta, where the O. longistaminata populations can cover several square kilometers, it is mainly O. breviligulata which invades the rice fields during cultivation. Once the rice fields are free, O. longistaminata takes over the ground again. The annual form survives in dense patches before disappearing completely within a few years.

Although the two species are frequently found side by side, the annual species seems to have a selective advantage in habitats that are disturbed and enriched.

O. longistaminata has a high fertility rate (> 50 %) in the large populations in the Niger Delta where the grain is occasionally harvested for consumption. In the isolated populations, however, the fertility rate tends to drop, although exceptions must be mentioned, especially a homogeneous population which invaded an abandoned rice field in Senegal and had normally fertile, small-sized plants.

Destruction of seed by a form of smut further increases the sterility of some populations which reproduce vegetatively. All the plants observed have rhizomes which seem capable of surviving in the soil during the dry season. In the Niger Delta, the soil is tilled after the flood has subsided in order to destroy them. The soil is ploughed up and then in the following season the young plants are cut. Most of the rhizomes are destroyed and the young plants, that have grown after the first rains and have been cut, are suffocated by the rapidly rising flood.

Although the rhizome character is constantly found, many characters have been observed to be highly variable. The anthocyanic colour of some organs such as the awns, pericarp, caryopsis and leaf sheath varies within the same population. The intermingled clones show how extensive vegetative reproduction is. On the whole, a high degree of intra- and inter-population variability occurs in characters such as grain size, leaf width, panicle size, etc.

At germination, albino or weak aberrant types occur fairly frequently, which indicates a heavy gene load. Many interspecific hybrids can also be found in the progeny of sympatric populations.

2 - Study of reproductive barriers

Reproductive barriers which isolate O. longistaminata from the other species of the sativa group with which they share genome A have already been described (CHU, MORISHIMA and OKA 1969, CHU and OKA 1970a, 1970 b). After recapitulating the main elements, we shall interpret some field observations on these lines.

According to the authors mentioned, breakdown in the free movement of genes between the autogamous and allogamous sections of the sativa series occurs at different levels, and in particular involves :

- Deterioration of F1 albumen
- Weakness of F1 generations
- Sterility of F1 embryo-sacs and especially microspores.

Deterioration of albumen is only found in crosses involving O. longistaminata. Weakness of the F1 generations and gametophytic sterility, on the other hand, occur in crosses involving the different forms of O. perennis.

These reproductive barriers isolate the perennial forms from the annual forms much more effectively in Africa than in Asia. Intermediate forms between O. Breviligulata and O. longistaminata have been looked for but never found. In Asia, however, (CHU and OKA, 1967) there is a continuous range of variation from the typically perennial to the typically annual forms.

Deterioration of the albumen, which appears in crosses with O. longistaminata in fact results in the loss of at least 97 % of the embryos formed. Of those that develop normally up to germination, about 50 % produce weak plants. A more or less high degree of gametic sterility then appears in the F1 plants obtained, but it may drop in selfed or backcrossed progeny. It has been established that introgression is possible between the autogamous and allogamous sections, but the possible exchange rate seems to be very low in Africa. Exchange seems to be easier between O. sativa and O. longistaminata than between the latter and O. glaberrima.

Deterioration of the albumen is controlled by a pair of complementary dominant lethal genes that have been called D1 and D2 by the above-mentioned authors. This pair of genes acts differently in reciprocal crosses according to their respective dosage in the triploid albumen.

CHU and OKA (1970 b) have shown that the O. longistaminata populations can be heterozygous for the two alleles of gene D1. Hybrids, called "obakes", have been found with higher fertility than O. longistaminata in crosses with the autogamous species. The "obake" forms therefore represent "interspecific bridges".

In the light of these bibliographical data we turned our attention to the complex plurispecific populations in the field.

Many large masses of crosses between O. breviligulata and O. glaberrima have been observed, as well as many plants, both isolated and in small groups, thought to be hybrids between O. saliva and O. glaberrima or O. breviligulata, but only the crosses with O. longistaminata will be dealt with here.

In Senegal, samples have been taken of three complex plurispecific populations presumed to involve introgression by O. longistaminata into cultivated rices. Several isolated plants thought to be hybrids between O. longistaminata and O. sativa have also been found. One is fertile. Three plants that are apparently hybrids between O. longistaminata and O. glaberrima have so far been recorded in the progeny of O. longistaminata collected from the edges of rice fields. These plants are weak, without rhizomes and have very small panicles and very irregular-sized sterile pollen grains. The presumed male parent has been determined from the acid phosphatase, peroxydase and esterase zymograms.

Other cases can certainly be found among the plants collected and they will be studied by electrophoresis analysis, examination of discriminating phenotypic characters and gametophytic fertility as well as in crosses with the different species studied.

These populations that have been sampled confirm that the introgression described by CHU and OKA exists in the field. Occasional introgression also seems, as stated in the bibliographical data, to be more frequent with O. sativa, which was introduced relatively recently, than with the annual African forms.

O. longistaminata also has another specific reproductive barrier, its self incompatibility (CHU, MORISHIMA and OKA, 1969).

In experiments we have checked its existence in the field by bagging panicles after flowering. It seems to be the rule in some populations in the Niger Delta which are otherwise fertile and have a high degree of intra-population variability. On the contrary, some populations from Senegal show average fertility when self fertilized.

Self-incompatibility therefore seems to be counterselected in small populations whereas it is the general rule in large populations.

The cause of sterility in some populations would seem rather to lie in the absence of intercompatible lines in these vegetatively reproduced populations than in gametophytic sterility.

3 - Analysis of enzyme variability

Some populations of O. longistaminata from the Mali and Senegambian centres have been analyzed by electrophoresis to compare them with the autogamous species (SECOND, BEZANCON, TROUSLOT, in preparation). We shall give the main results concerning chiefly the qualitative aspect of enzyme variability.

150 individuals of O. longistaminata have been studied. To compare them with the Asian branch of O. perennis, a few representatives of the perennial, intermediate and annual forms of this species (W 036 - W 107 - W 120 - W 133 - W 149 - W 157 - W 163 - W 630 - W 1183 - W 1185 - W 1236 - W 1294) were analyzed, together with a few representatives of the intermediate perennis-sativa forms from the JEYPORE area in India (samples kindly supplied by Dr. OKA).

a) Esterases : The esterase zymograms of O. longistaminata are extremely complex. From one individual to another, many bands appear at different migration distances. The probably high heterozygosis further increases the number of bands while at the same time reducing the activity of some. Overlapping occurs frequently. For all these reasons, statistical interpretation of zymograms in band frequency is difficult for esterases.

Figure 2 shows a few examples of the variability observed with the O. breviligulata (esterase A^{+-} type) and O. sativa (esterase 2 type) checks. The letters correspond to the different standard bands of O. breviligulata and are used as references. The zymograms given have been chosen to show the types of variability that exist for the main bands but the number of combinations that can be observed is far higher.

The differences compared with the autogamous species involve the following three cases :

- band B : this is generally present at the same distance as in the autogamous species but it may appear at different distances and even disappear completely. In all the autogamous lines it is a single band but it may disappear in O. sativa.

- band E : only one individual has been found to have this band at the same distance as in the autogamous species: it generally appears at different distances. In the autogamous species, it may occur at two different levels and may disappear.

- band D : this one has never been found in O. longistaminata. A similar band (a double red one) has however been observed in some individuals but at much greater migration distances (similar to band K). It exists at 3 different but similar levels in O. breviligulata but disappears in O. sativa.

The Asian samples of O. perennis seem to be much closer to the autogamous species than O. longistaminata, except for N° W 1294 (perennial type from the Philippines). Bands E and D are in fact frequently found in them.

Although, from the species standpoint, the variability of O. longistaminata is enormous compared with O. breviligulata, there are great differences in individual richness in terms of the number of bands. O. breviligulata appears to be moderately rich.

b - Peroxydases : Figure 3 shows the zymograms observed using young leaves.

For the fast anodic band, all the O. longitaminata observed are seen to be identical to O. sativa. Only O. breviligulata and O. glaberrima have slightly slower bands.

Table I gives the distribution of the different types observed in 3 populations from the Niger Delta in Mali.

Table I - Frequencies of peroxydase zymograms in three populations from Mali

Zymograms Populations	LI	LII	LIII	LIV	LV	LVI	LVII
L L 9	15	6	3	5	-	1	-
L L 10	11	11	3	14	6	3	1
L L 11	11	2	-	3	3	-	-

c - Dehydrogenase malates. Figure 4 shows the different zymograms that can be observed in a single population of O. longitaminata from the Niger Delta, whereas for the 3 autogamous species together there is only a single zymogram of medium complexity.

Table II gives the frequencies of the different zymograms observed in 3 populations. The zymogram of the autogamous species is found to be by far the most frequent for O. longistaminata which seems to vary around this average figure.

All the Asian samples of O. perennis analyzed have zymogram 1.

Table II - Frequencies of the dehydrogenase malate zymograms in three populations from Mali.

Zymograms Populations	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Tota
L L 9	13	6	7				1	1	1		3	32
L L 10	46	7	19	6	1		2			1	2	84
L L 11	24	4	2		1						1	32

d - Acid phosphatases

This enzyme has been studied by the method described by PAI, ENDO and OKA (1975). We have used these authors' symbols to describe the variability observed which is given in figure 5.

Group AMC

The following different mobilities have been observed: +4, + 9, + 12, hybrid + 4 and + 12, hybrid + 9 and + 12 with frequencies of 0.80, 0.03, 0.09, 0.06 and 0.01 respectively (64 individuals).

The + 4 individuals were then classified according to + 4 and + 6 mobility which forms a new category.

It is worth mentioning that + 9 mobility which is a characteristic feature of O. breviligulata and the Japonica type of O. sativa is rare for O. longistaminata.

Band Fa

O. breviligulata does not have this band. It is only found in 50 % of the O. longistaminata individuals observed and has slight mobility differences: a frequently occurring + 45 mm type, which is a characteristic feature of the Indica types of O. sativa, and a rare + 48 mm type.

Bands B and b

This band, which is a characteristic feature of O. longistaminata, has invariably been found, with 2 mobilities: approximately - 21 and - 26, with frequencies of 0.91 and 0.02 respectively, the remainder being hybrids of the two forms.

e - Amino-peptidase leucines (fig. 6).

This enzyme is extremely variable in O. longistaminata which has more bands than the autogamous species with the same but often less intense bands. It is strange to find that it is apparently the bands with average loads that have been retained in the autogamous species.

f - Overall analysis and population structure

For band frequency, the enzyme variability of O. longistaminata generally seems to be centred around that of the autogamous species. There are however differences for the most variable enzymes, particularly esterases for which the O. longistaminata variabilities do not cover those of the autogamous species.

As for population structure, a single O. longistaminata population is seen to contain a far higher degree of enzyme varaibility than the three autogamous species together. This is mainly true for the large heterogenous populations in the Niger Delta, since the small marginal populations naturally tend to be more homogenous.

Heterozygosis of individuals can be seen in zymograms with two allelic bands but also when the progenies of the same plant are compared. These data will have to be assessed quantitatively when genetic determination of the zymograms is better understood.

4 - Discussion and hypotheses

The high enzyme variability of the allogamous section of O. longistaminata compared with the lower convergent variability of the African and Asian autogamous sections is linked with far greater tolerance of varied ecological conditions. Adaptation to varied environmental factors can be demonstrated in experiments on the sensitivity to heat of germinating seeds: O. longistaminata has an intra-population variability which enables some individuals to develop at extreme temperatures which are not supported so well by autogamous species (unpublished preliminary data).

The adaptability of O. longistaminata does not therefore seem to be limited to spatial variations in the environment within the species' distribution area. It may extend to time variations encountered by the species during its evolution.

The theory of fitness in a structured environment (LEVINS, 1965) explains this heterozygosis in a heterogeneous environment. An allele may be dominant in a population while at the same time allowing a great deal of variation around this optimum.

BRYANT (1974) also shows that the temperature factor may largely explain enzyme variability particularly in poikilothermal organisms. Experiments are being undertaken to check whether the heterogeneity of the O. longistaminata populations with regard to their responses to temperature gradients is due to their enzyme variability.

O. longistaminata which has, often with high frequency, the zymogram bands observed in the autogamous sections and linked with many variants, may be thought to be the African pool of genome A variability.

The fact that some of the zymogram bands of the autogamous species are not found in O. longistaminata may be explained by the existence of reproductive barriers which isolate the allogamous series in Africa. These reproductive barriers have enabled O. breviligulata to colonize specifically habitats that are disturbed and enriched by animals, while at the same time maintaining occasional exchanges with O. longistaminata.

In Asia, where introgression between autogamous and allogamous species is more frequent, these zymogram bands, which are characteristic features of the autogamous sections and do not occur in O. longistaminata, are apparently sometimes found in the allogamous perennial forms. Our data on this subject are however restricted to a very small sample which nevertheless includes intermediate, perennial and annual forms.

The reproductive barriers described may therefore be interpreted as filters of gene flows selected at adjusted levels by the species (PERNES, SAVIDAN and RENE-CHAUME, 1975). Due to the homozygosis of the autogamous series, from the total variations in the pool, only the variations strictly suited to their habitat conditions are retained; hence the convergence of variability in the African and Asian autogamous series. This convergence appears in wild populations of O. breviligulata and the intermediate O. perennis-O. sativa forms in the JEYPORE area in India. It increases during domestication when they acquire stricter autogamy which is linked with a man-controlled environment.

The acquired autogamy and domestication also result in very considerably reduced enzyme variability.

The more advanced avolution towards cultivated types is, the simpler the zymograms seem to be. Thus the evolutive differentiation of the O. sativa species is mainly due to the loss of bands in one line or another rather than to differen band migration distances from one individual to another. Similarly, during the evolution of O. glaberrima, the simplest esterase zymograms of O. breviligulata seem to have been retained. Nevertheless, O. glaberrima is still relatively rich in isozymes compared with some O. sativa lines.

As a hypothesis, it is suggested that differentiation of rice into ecotypes involves the loss of some isozymes. Isozymes that are useless in a given environment may be lost but complementary semi-lethal recessive genes which favour genetic separation into two ecotypes may also be formed.

This interpretation concurs with the generally accepted genetic causes of F1 pollen sterility and the breakdown of F2 structures: complementary lethal recessive genes (OKA, 1962 and 1974). This also explains the complementing found in hybridization between distant lines with returns to ancestral characters: aristation, seed-shedding, opening of panicles, etc.

Another working hypothesis is that there is a strong connection between the adaptability of a line and its enzyme richness in individuals.

The existence of such a relation would be extremely important in research into lines with good adaptability.

MORISHIMA and OKA (1975) have shown that the plasticity of the perennial types is very high with regard to the sizes of different organs when compared with the cultivars. Cultivars however seem to respond better to mineral nutrition for the number of panicles per plant.

The Oryza genome A gene pool in Africa therefore seems to be divided into two types of sections according to the lay-out and terminology defined by PERNES, SAVIDAN and RENE-CHAUME (1975).

a - a pool section : O. longistaminata. The perennial and self-incompatible characters, which are linked with vegetative reproduction, enable it to bear a heavy gene load (alleles that are silent or have little adaptation value) in the present environment. The high degree of heterozygosis preserves many variants in addition to the best adapted dominant allele that is often retained in the autogamous series. This pool section preserves the variability left by the ancestral form before the break-up of the continents and the variability acquired since. This variability enables it to survive slow environmental variations but it develops best in an environmant free of sudden changes: regularly flooded areas in the Niger Delta for example.

b - colonization sections : the acquired autogamy leads them to converge towards a lower degree of variability suited to a particular environment. The resulting lighter gene load gives them a high reproduction potential which enables them to spread rapidly in a scattered migrant habitat. With differentiation into ecotypes many silent alleles are fixed, which results in poorer enzyme resources and reproductive barriers caused by the formation of pairs of complementary lethal genes.

This brings up the question of the real significance of this shedding of active alleles: is it selection or drifting ?

The variability of these sections, which are isolated from one another by a reproductive barrier resulting from their relative differentiation, seems to be due mainly to introgressions into the allogamous pool. Autogamy polarizes this exchange towards the allogamous pool. and is a threat to its existence.

In Africa, a "gene flow filter" seems to have been formed which has the following two effects :

- preservation of high heterozygosis in the allogamous pool which makes it possible to store a substantial degree of allele variability,

- a tendency to reject O. longistaminata from the restricted ecological site of O. breviligulata.

These two sections therefore form two independent entities that are nevertheless connected. The two together make up the overall pool of variability for cultivars in Africa.

By studying populations where the two species exist side by side it will be possible not only to sample their respective variability but also gain a better understanding of how they are connected and observe any introgressions that may occur.

Nevertheless, to collect maximum allele variability, it is better to use the large populations of O. longistaminata which retain maximum heterozygosis.

Within this mechanical system, the value of going back to wild pools seems obvious. Particular mention should be made of :

- repeating the domestication process in order to obtain transgressions and colonize new ecological areas that can be cultivated;

- acquiring sources of plasticity or resistance for the adaptability of cultivated varieties;

- introducing new characters in cultivated varieties: self-incompatibility for example.

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Fig. 1 - Estérases: Différents zymogrammes observés parmi les espèces autogames *O. glaberrima*, *O. breviligulata* et *O. sativa*.

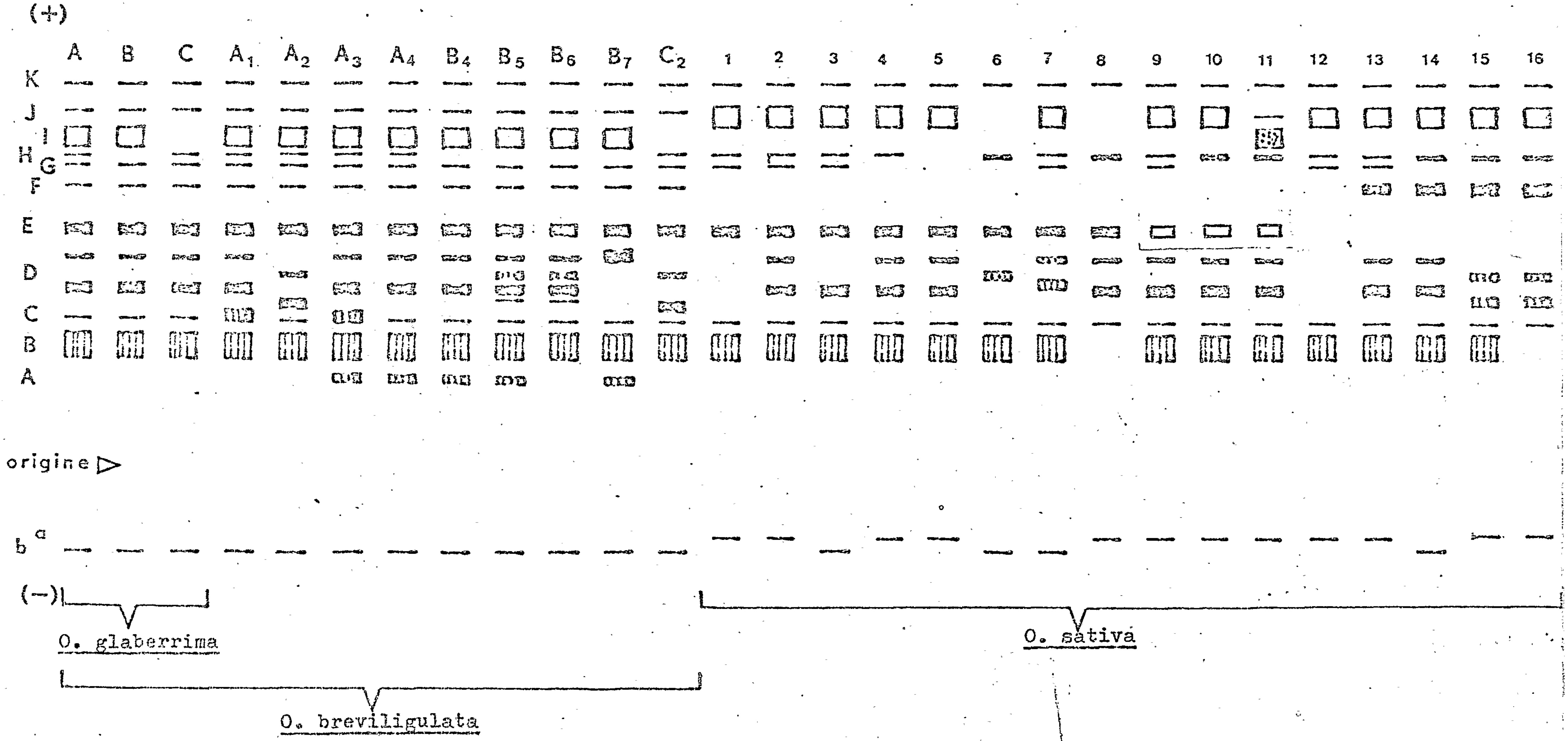

Fig. 2 - ESTERASES : Quelques zymogrammes d'*O. longistaminata* choisis pour représenter la variabilité observée au niveau des bandes principales. Le nombre de zymogrames différents est beaucoup plus important. A gauche du diagramme, on a placé 2 témoins: *O. breviligulata* (A_3) et *O. sativa* (2) et à droite, les zymogrammes observés parmi un échantillonnage d'*O. perennis* asiatique et de formes intermédiaires entre *O. perennis* et *O. sativa*.

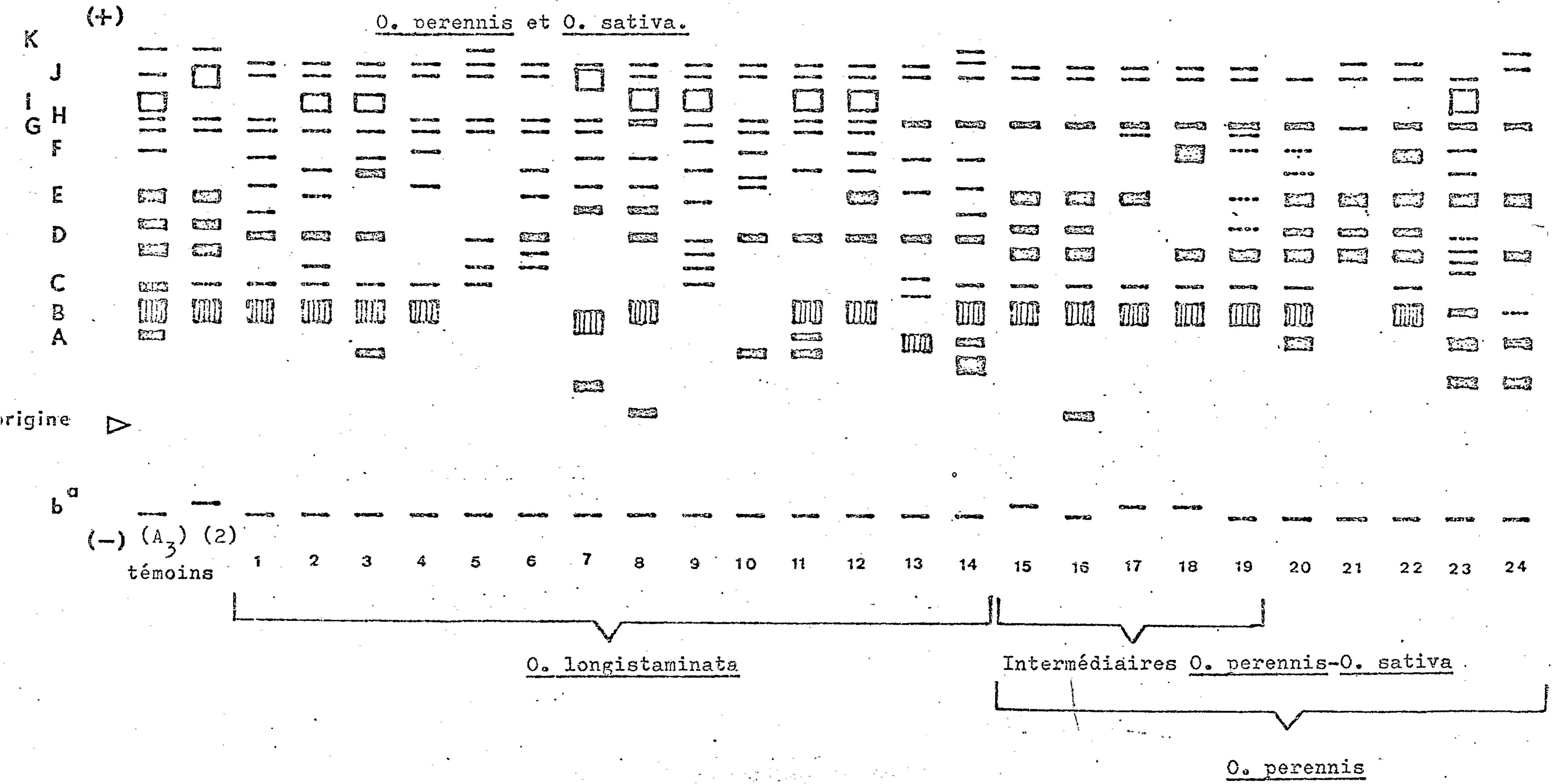

Fig. 3- PEROXYDASES; différents zymogrammes observés par èspèces.

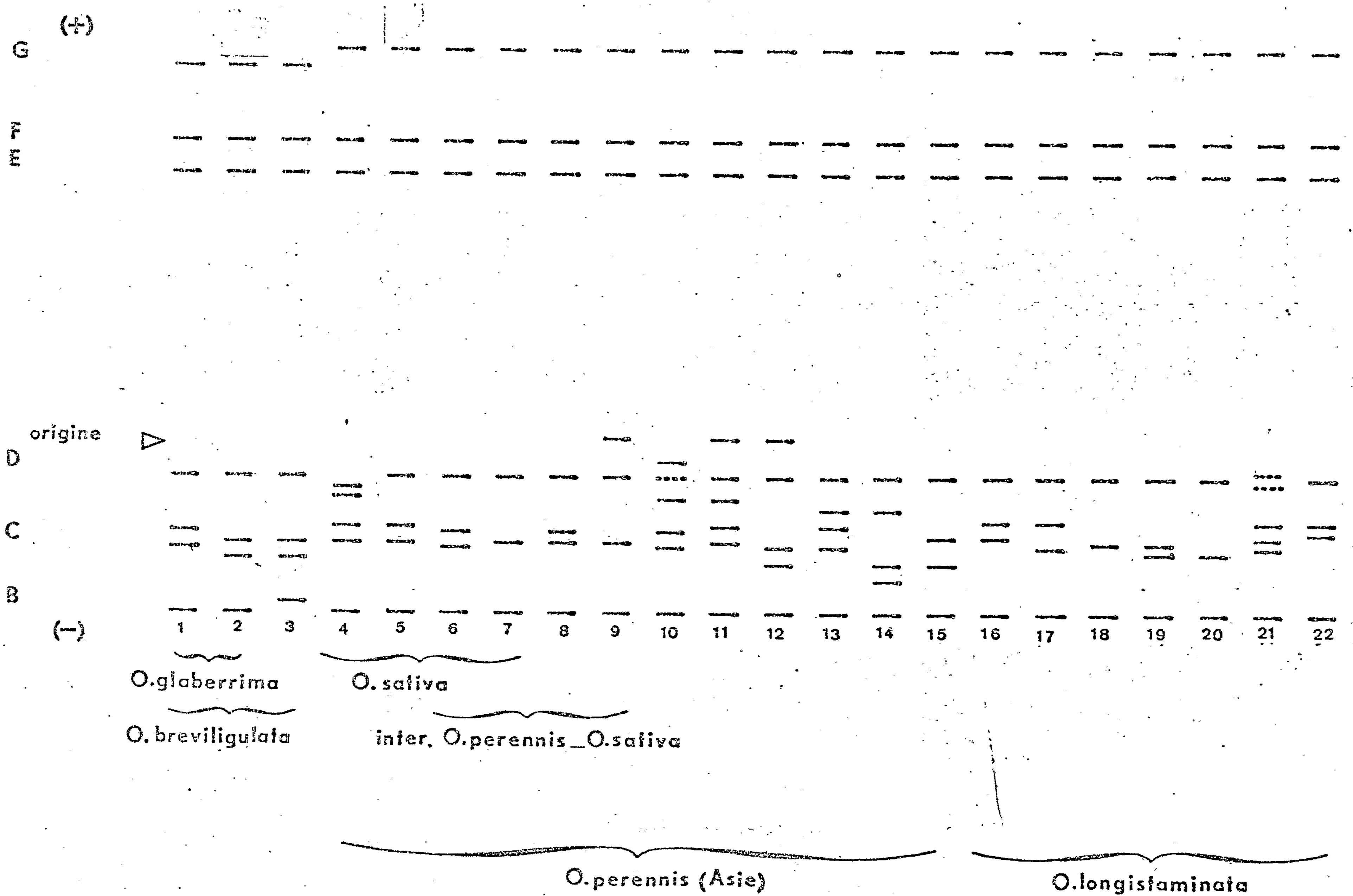

Fig. 4 - MALATE DESHYDROGENASES : Différents zymogrammes observés, par espèce.

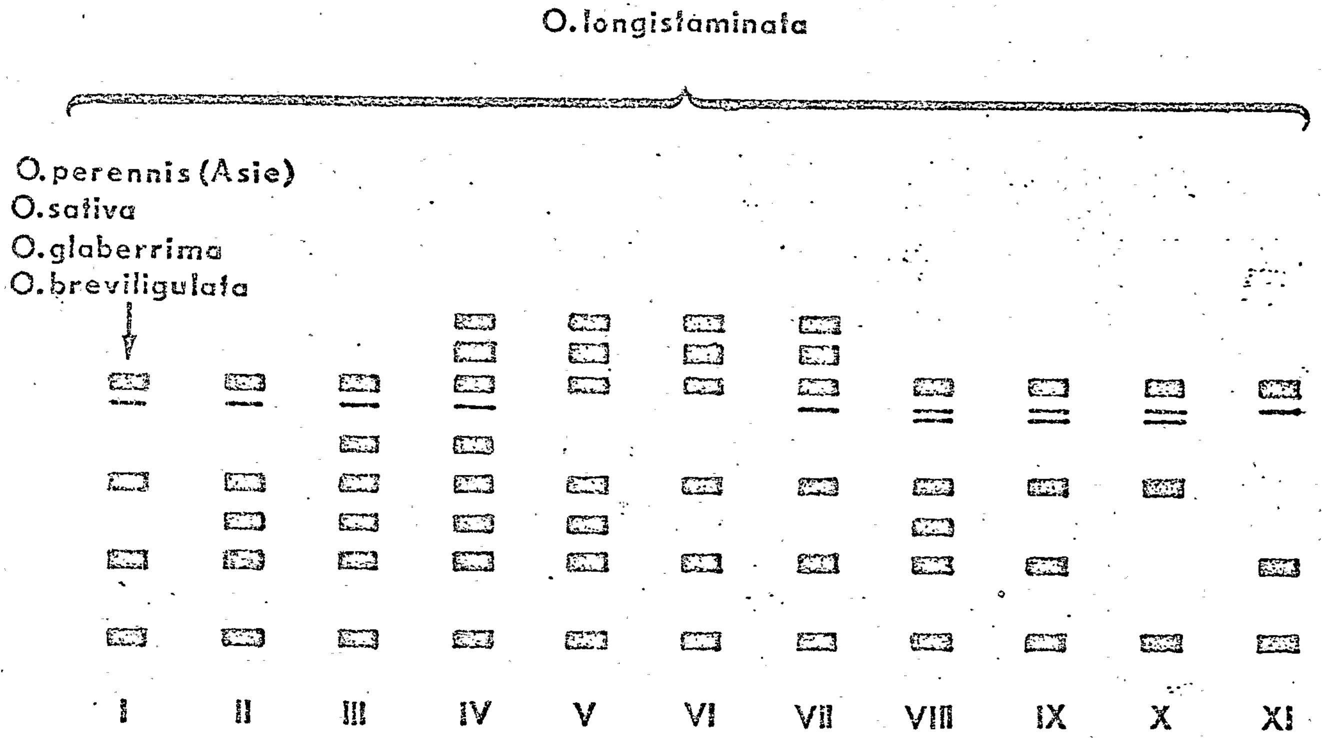

Fig. 5 - PHOSPHATASES ACIDES : Variabilité observée au niveau des différentes bandes, par espèce (tous les zymogrammes d'<u>O. longistaminata</u> observés n'ont pas été représentés).

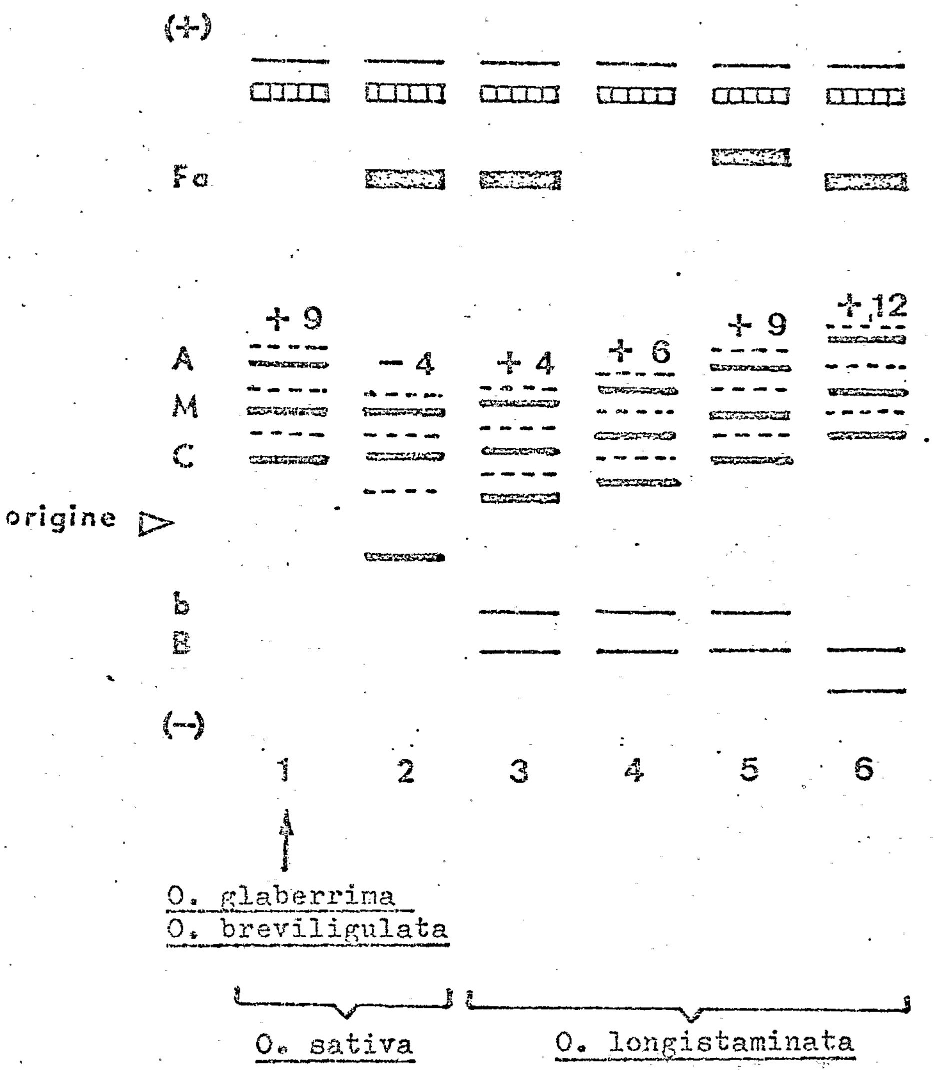

Fig. 6 - LEUCINE AMINO-PEPTIDASES : Différents zymogrammes observés par espèce.