

around 280. However, some bands seem at first sight to be 'hot spots' of mdg-1 insertion (see fig. 2 and the tail of the distribution in fig. 3). This is particularly striking for the bands 19F, 39CD, 42A, 48E, 51D, 98C. Some of them were also shown in other experiments to be sites of insertions or hot spots for transposition^{5,12,14}. The number of these hot spots, however, is small and thus the distribution in figure 3 does not deviate significantly from a Poisson distribution. The hot spots may be clusters of mobile elements^{15,18,19} and their location may characterize a population. This hypothesis is reinforced by the observation that among the sites that hybridized most frequently in our experiment with a natural population are some that also hybridized most frequently in previous experiments with laboratory populations¹⁴.

To test whether the different distribution spectra of insertions are correlated with different classes of viabilities, we analyzed the rough data by a factorial correspondence analysis²⁰. Naturally, we had only a contingency table with the values 0 and 1 (absence or presence of an insertion site in a bands), but factorial correspondence analysis is the best and most powerful technique that can be used to analyze such a table. The aim of this method is to represent the data in a new space with a reduced number of dimensions. Figure 4 represents the projection of the values on the plan of the second and third components of the analysis. We choose these two plans as they lead to a better discrimination of the different families; the larvae from the natural population are dispersed on the second and third axes of the analysis but not on the first one. As seen from figure 4, the different classes of viability progenies have distinct frequency spectra of mdg-1 location. For example the correspondence analysis reveals that a pattern with insertions in bands 7D, 38C, 51B, 56E, 67C, 75F, 76B, 85B, is seen only in families from the low egg hatchability class (that is also the class with the lower values of egg-to-adult survival).

It is well known that differences in the location of mobile elements occur among individuals from the same population. Our data indicate in addition that the pattern of distribution of mdg-1 may correlate with the viability value of the family from which the larva comes. However, in situ hybridization was done on chromosomes of inbred larvae that survived. These larvae, as seen from figure 4, may have a particular pattern of mdg-1 locations. It is possible that the embryos and larvae that dies had another particular distribution spectrum of mdg-1 locations connected with lethality. This hypothesis is reinforced by recent results showing that a specific mobile element, the L factor, may be involved in the lethality associated with an unstable X chromosome²¹.

Many experiments will be necessary to be able to assess the role of mobile elements in natural populations. Our results indicate

that the search for relationships between location of elements and viability or other characteristics associated with fitness should be a promising way of investigation.

Acknowledgments. We thank V. Gvozdev for useful discussion, M. Ashburner for critically reading an earlier draft of the manuscript, L. Konovalchouc for valuable technical assistance, P. Fouillet, E. Wajnberg and D. Pontier for help with statistics and computerization. This research was supported by the USSR Academy of Sciences and the Centre National de la Recherche Scientifique (Laboratory No. 243).

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0014-4754/85/111475-03\$1.50 + 0.20/0

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Occurrence of ricinoleic acid in submerged cultures of various *Claviceps* sp.

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Summary. Ricinoleic acid was found in different *Claviceps* sp., indicating that it is neither specific chemotaxonomic marker of *Claviceps purpurea*, nor a specific indicator of peptide alkaloid synthesis.

Key words. *Claviceps*; ricinoleic acid; ergot alkaloids; fatty acids.

Mycelial differentiation of the fungus *Claviceps* involves morphological and biochemical changes. In parasitic cultures a conspicuous feature of differentiation is the formation of sclerotium and alkaloid synthesis. An analogous pattern is found also in saprophytic *Claviceps* strains. In these strains the production of alkaloids under conditions of submerged cultivation is accom-

panied by sporulation² and differentiation of the culture into the sclerotial type of mycelium³. Sclerotial cells are assumed to be the dominant producers of alkaloids^{3,4}. Another indicator of differentiation of the culture into the sclerotial type is abundant production of lipids (triglycerides)⁴⁻⁶.

In sclerotial cells lipids are found in substantially higher

amounts than in cells of a sphaelial mycelium and are unique in that they contain ricinoleic acid ([R-(Z)]-12-hydroxy-9-octadecenoic acid). Ricinoleic acid has so far been considered an important biochemical indicator of morphological and biochemical differentiation of *C. purpurea* mycelium. Its level reaches a maximum closely before the onset of peptide alkaloid formation⁷ and exceeds the mean level found during fermentation by as much as 49%⁷. High-producing strains of *C. purpurea* contain in their lipid fraction up to 40 mol% ricinoleate⁵. Total cell fatty acids show an analogous pattern during fermentation⁷. In contrast to production strains of *C. purpurea*, nonproduction strains contain no ricinoleic acid and their content of total lipids is substantially lower⁵. Biosynthesis of ricinoleic acid and ergot peptide alkaloids probably have a common regulatory mechanism^{5,6}. Among the *Claviceps* sp. under study, i.e. *C. purpurea*, *C. paspali* and *C. sp. SD-58* (probably *C. fusiformis*)⁶, the relationship between mycelial differentiation and the occurrence of ricinoleic acid was proved only in *C. purpurea* producing peptide alkaloids⁶. These findings formed the basis of the hypothesis that ricinoleic acid is a specific chemotaxonomic marker of *C. purpurea* strains producing peptide alkaloids⁶.

We studied the content of fatty acids in submerged cultures of six species of *Claviceps* deposited in the collection of this institute: *C. purpurea* 129/35, *C. purpurea* PLA 4, *C. fusiformis*, *C. sp. SD-58*, *C. paspali* MG-6, *C. paspali* FA (CCM F-731). Inoculation medium was T1⁸. A bioreactor (3 l) with medium CS2⁸ (1.5 l) was

inoculated with a 7-day-old submerged inoculum (120 ml). The paddle impeller speed in the bioreactor was 500 rpm, aeration rate 1 l/min, temperature 24 ± 1 °C. After 10-day cultivation the mycelium was collected by centrifugation and washed with water. Methyl esters of fatty acids were prepared by method No. 3 described by Marberry⁹. A mixture of the methyl esters was analyzed by gas chromatography-mass spectrometry on a HP 5995B (Hewlett Packard, USA) instrument on a capillary fused silica column under conditions described previously¹⁰. The content and composition of the alkaloid mixture was analyzed by high performance liquid chromatography¹¹.

In contrast to reports in the literature, ricinoleic acid was found in the mycelium of different *Claviceps* sp. (table) and is thus not a specific chemotaxonomic marker of *C. purpurea*, nor a specific indicator of synthesis of peptide alkaloids. In general, no significant correlation was found between the rate of production of alkaloids in different *Claviceps* sp. and the cellular level of ricinoleate. However, a valuable finding was that such a relationship was exhibited solely by *C. purpurea* strains producing peptide alkaloid and that the correlation between an increased level of ricinoleate in the mycelium and the differentiation of the mycelium to the sclerotial type was characteristic solely of the species *C. purpurea*.

Ricinoleic acid in mycelium of submerged cultures of various *Claviceps* sp. producing alkaloids

	Alkaloids Type	Production (μg/ml)	Ricinoleic acid*
<i>C. purpurea</i> 129/35	chano-I-, agro-, elymoclavine	1000–4000	1.90
<i>C. purpurea</i> PLA 4	peptides (cyclols)	7–10	3.24
<i>C. paspali</i> MG-6	lysergic acid	20–40	0.30
	α-hydroxyethylamide		
<i>C. paspali</i> FA	lysergic acid	1000	0.20
	α-hydroxyethylamide		
<i>C. fusiformis</i>	chano-I-, agro-, elymoclavine	1000–2000	4.39
<i>C. sp. SD-58</i>	chano-I-, agro-, elymoclavine	1000–2000	4.11

* Percentage of fatty acids mixture.

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0014-4754/85/111476-03\$1.50 + 0.20/0

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Biological properties of alkaloids. Influence of quinolizidine alkaloids and gramine on the germination and development of powdery mildew, *Erysiphe graminis* f.sp. *hordei*

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Summary. The quinolizidine alkaloids sparteine, lupanine and 13-tigloyl-oxylupanine and the indole alkaloid gramine inhibited the germination of conidia of *Erysiphe graminis* f.sp. *hordei* Marchal and also the further development to appressoria. Half-maximal inhibitory concentrations of these alkaloids were 1–5 mmol/l. This result provides further evidence for a role of alkaloids as chemical defense compounds in plants.

Key words. Quinolizidine alkaloids; gramine; powdery mildew; *Erysiphe graminis*; inhibition of germination.

There is sufficient evidence to assume that most of the so-called secondary products or allelochemicals of plants are not waste products, but dynamic metabolites which are important for the biological fitness of the plants. These compounds either serve to attract pollinating or seed dispersing animals or repel and inhibit herbivores and microorganisms^{1–11}. We have studied the bio-

logical properties of quinolizidine alkaloids (QA), common natural products of many Leguminosae, in this context^{12–17}. We concluded that a minor function of QA in lupins is nitrogen transport and nitrogen storage^{18,19}, but that their major function is chemical defense. It could be shown experimentally that QA deter herbivores (mammals, insects, snails) from feeding, and