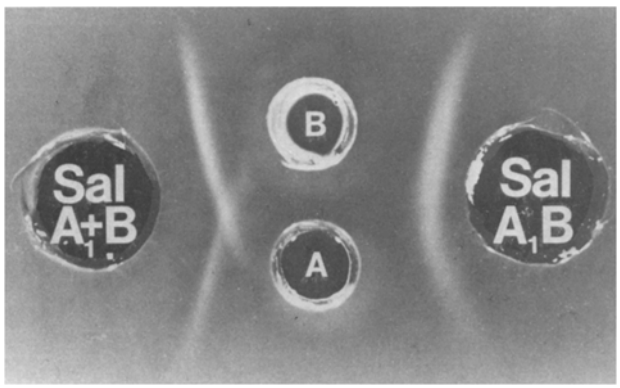

Figure 1. Double diffusion precipitin reactions of anti-A (A) and anti-B (B) antibodies against saliva (Sal) of an A_1B secretor individual and against a mixture of salivas of A_1 and B secretor individuals.

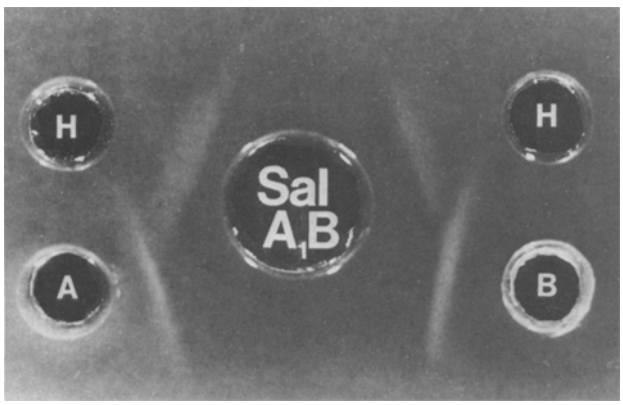

Figure 2. Anti-A and anti-B antibodies reacting besides anti-H (H) lectin against saliva of an A_1B secretor individual.

Figure 1 displays the precipitin pattern obtained from anti-A and anti-B antibodies reacting with saliva from a group A_1B secretor individual and a mixture of salivas from group A_1 and B individuals. The mixture of A_1 and B salivas exhibits bands of non-identity (bands crossing) while the A_1B saliva shows a pattern of identity (smooth continuation). These patterns provide visual evidence that in the A_1B saliva the A and B antigens are situated predominantly on the same molecule. All 7 A_1B salivas tested formed continuous precipitin bands between anti-A and anti-B antibodies.

Precipitin band formation of anti-A and anti-B antibodies placed in wells beside anti-H lectins is shown in figure 2. The patterns exhibit either partial identity or non-identity suggesting that in saliva of A_1B individuals the H antigen is primarily situated on molecules other than the A and B antigens. The figure shows relatively weak and diffuse bands against anti-H lectin, indicating relatively low concentration of H substance in the A_1B saliva. By hemagglutination inhibition tests, the mean concentration of H substance was reported to be lower in saliva of AB individuals than in those with blood group O, A_1, A_2 or B[5].

The band of identity formed against the A_1B saliva, displayed in figure 1, provides visual evidence that the A and B antigens are primarily situated on the same molecule. This may well be the first example of such a pattern of identity against soluble molecules with known different antigens, although it has been previously reported for the cucumber mosaic virus[6]. However, this virus is much larger than the soluble substances of saliva. Indirect evidence that the A and B antigens are on the same molecule has been previously reported[7,8]. The synthesis of soluble substances possessing predominantly both the A and B antigens requires a mechanism that adds regularly D-galactose and N-acetylgalactosamine to the same molecule. The previously reported finding of a hybrid glucosyltransferase in A_1B individuals[9], can explain the resulting band of identity (fig. 1). The foregoing figures further indicate that the soluble substances from A_1B individuals predominantly carry either the H or A and B, but not all three antigens.

Vitala et al.[10] reported that the A and B antigens of AB erythrocytes are located on different glycopeptide chains. This points to a possible difference in the arrangement of the A and B antigens of erythrocytes and the soluble substances. However, Vitala and coworkers digested erythrocyte membranes with pronase, obtaining blood group reactive glycopeptides with a mean mol.wt of 10,000. This is at least 20 times lower than the molecular weight of blood group substances[11]. Consequently, the question arises whether the glycopeptides after pronase digestion of membranes still represent the original antigen organization or whether this arrangement was broken.

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Genetic control of malate dehydrogenase in *Nicotiana suaveolens* and *N. glutinosa*

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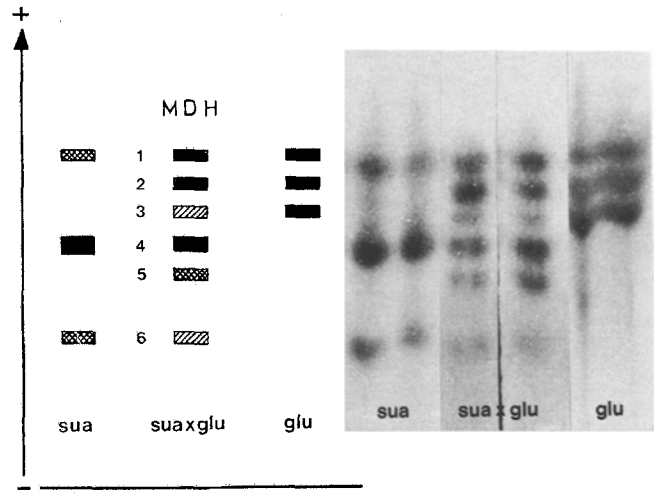
Summary. The study of malate dehydrogenase patterns in leaves of *Nicotiana suaveolens, N. glutinosa* and their interspecific hybrid has been carried out, in order to propose a model of its genetic control. Two possible explanations for the genetic control of malate dehydrogenase have been postulated for *N. suaveolens,* and at least two loci appear to be implicated in the control of this system in *N. glutinosa.*

Key words. Nicotiana suaveolens; N. glutinosa; interspecific hybrid; malate dehydrogenase.

There are several investigations which have used isozyme systems to analyze morphogenetic processes induced by plant tissue culture in *Nicotiana* species[2–6]. One of these isozyme systems was malate dehydrogenase[5], and according to Scandalios[7], malate dehydrogenase can be used as a marker for differentiation. For these reasons the knowledge of the genetic control of malate dehydrogenase must be of interest, above all in the case of *Nicotiana* species which are much used in plant tissue culture. The study of genetic control of malate dehydrogenase in *Nicotiana*, as well as of other isozyme systems, presents serious difficulties due to the absence of variability[8,9]. This fact made it necessary to use methods different from the ones of formal genetics[9,10]. In this paper, the genetic control of malate dehydrogenase in *N. suaveolens* and *N. glutinosa* (both species used in plant tissue culture) is described, following the same method employed in the same species for two other isozyme systems[9]; analyzing the isozyme patterns of the interspecific hybrid in comparison with the ones of the parental species.

Material and methods. Two species of *Nicotiana, N. suaveolens (sua)* and *N. glutinosa (glu)* were used. Seeds were obtained from different places[9]. In all cases, the plants analyzed showed the same pattern, and no intraspecific variability was found. The interspecific hybrid (*sua* × *glu*) was obtained by crossing *sua* from Instituto Tecnológico del Tabaco de Sevilla, and *glu* from Hortus botanicus Bergianus from Stockholm. Isozyme analyses were carried out with a crude extract of leaves of flowering plants, using 0.043 M tris-citric acid, pH 7.0 as electrode buffer, and 0.006 M histidine, pH 7.0 as the gel buffer (12% starch). The electrophoresis was carried out at 150 V and 45 mA/gel, during 5 h, in darkness at 4 °C. The staining procedure was that of Shaw and Prasad[11].

Each locus has been called by the name of the isozyme system, and numbered in order from the faster to the slower mobility. Each allele has a subindex added to the locus name, as well as a letter, g or s, to indicate whether it corresponds to *glu* or *sua* respectively.



Electrophoretic banding patterns of MDH in *N. suaveolens, N. glutinosa* and their interspecific hybrid.

Results and discussion. Three bands were observed, namely 1, 2 and 3, which constituted the MDH pattern of *glu* (fig.). The band numbers 1, 4 and 6 were present in *sua*. The pattern of the hybrid showed the 5 bands already indicated, plus a new band, number 5, which did not appear in the parental patterns.

The appearance of additional bands in the hybrid would indicate the dimeric structure of MDH isozymes. So, band 5 would be the heterodimer between bands 3 and 6. The dimeric character of

Malate dehydrogenase system (MDH)
A) MDH genes involved in each genome

	Species	*sua*		*sua* × *glu*			*glu*
	Genomes	S_1	S_2	S_1	S_2	G	G
1a	MDH-1 genes	MDH-1_{1s}	mdh-1_s	MDH-1_{1s}	mdh-1_s	MDH-1_{1g}	MDH-1_{1g}
	Subunit coded	α	–	α	–	θ	θ
	MDH-2 genes	mdh-2_s	MDH-2_{2s}	mdh-2_s	MDH-2_{2s}	MDH-2_{1g}	MDH-2_{1g}
	Subunit coded	–	γ	–	γ	β	β
1b	MDH-1 genes	MDH-1_{1s}	mdh-1_s	MDH-1_{1s}	mdh-1_s	MDH-1_{1g}	MDH-1_{1g}
	Subunit coded	α	–	α	–	θ	θ
	MDH-2 genes	MDH-2_{2s}	mdh-2_s	MDH-2_{2s}	mdh-2_s	MDH-2_{1g}	MDH-2_{1g}
	Subunit coded	γ	–	γ	–	β	β
2	MDH-1 genes	MDH-1_{1s}	MDH-1_{2s}	MDH-1_{1s}	MDH-1_{2s}	MDH-1_{1g}	MDH-1_{1g}
	Subunit coded	α	γ	α	γ	θ	θ
	MDH-2 genes	mdh-2_s	mdh-2_s	mdh-2_s	mdh-2_s	MDH-2_{1g}	MDH-2_{1g}
	Subunit coded	–	–	–	–	β	β

B) Theoretical intensities of *sua* × *glu* bands

Band	Subunit	Theoretical intensities
1	$\alpha\alpha$ $\alpha\theta$ $\theta\theta$	4
2	$\alpha\beta$ $\theta\beta$	4
3	$\beta\beta$	1
4	$\alpha\gamma$ $\theta\gamma$	4
5	$\beta\gamma$	2
6	$\gamma\gamma$	1

1a and *1b* Two possibilities for the hypothesis 1. *2* Hypothesis 2. mdh-1_s and mdh-2_s are non active, null alleles. MDH-1 and MDH-2 genes for *sua* and MDH-1 and MDH-2 genes for *glu* are related, belonging to the same gene family, although not necessarily allelic.

MDH has been described in other plant species such as maize, barley and rye[12-14].

Since all the *glu* plants analyzed have the same 3 banded pattern, it could be concluded that *glu* plants are homozygous, and therefore, in diploid species, such as *glu*, at least 2 loci are needed to obtain 3 isozymes. *Glu* plants must be homozygous MDH-1_{1g} MDH-1_{1g}, MDH-2_{1g} MDH-2_{1g}, the fastest migrating subunit being coded by the MDH-1_{1g} allele and the lowest by the MDH-2_{1g}, isozyme 2 being the heterodimer.

Two possible hypotheses can be postulated for the explanations of the *sua* pattern, admitting its fixed heterozygous nature, due to the fact that it is an amphiploid species[9]:

1) The 2 loci are represented in its genomes, but only active alleles, MDH-1_{1s} and MDH-2_{2s}, are present (table).

2) Only 1 locus is represented by active alleles, MDH-1_{1s} and MDH-1_{2s} (table).

Since the relative staining intensity of hybrid plant isozymes appears to fit a 4:4:1:4:2:1 pattern instead of a 9:6:1:12:4:4 pattern, it is necessary to postulate that the amphiploid species contributes to the hybrid only with 2 active gene doses instead of the 4 expected (see table). Therefore the *sua* species must be fixed for null alleles. An alternative explanation is that chromosome segments have been lost during the evolution of *sua*. According to Goodspeed[15] this species should have lost chromosomes or chromosome fragments until attaining the actual chromosome number. If this is correct, it could be possible that the loci MDH-2 and/or MDH-1 were lost in the first and/or second genome.

Another alternative hypothesis that cannot be ruled out is that the diploid species of *Nicotiana* had in their origin only 1 locus of MDH, and the 2nd MDH locus of *glu* came from a duplication; duplicated genes for MDH have been described in diploid species[13,16,17]. If this last hypothesis was true, it would not be necessary to postulate the existence of null alleles or chromosomal losses in *sua*.

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Gamete formation reflects the sexual pheromone hierarchy of *Dictyostelium giganteum*[1]

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Summary. Sexual development in *Dictyostelium giganteum* begins with the appearance of small, amoeboid gametes that fuse to produce mainly binucleate cells which differentiate into zygote giant cells. The data presented here show that the number of gametes produced by each strain (WS589 > WS606 > WS607 > WS588) is directly related to its position in this hierarchy.

Key words. Mating types; sex pheromone; gametes; zygote formation; *Dictyostelium*.

Sexual development in both homothallic and heterothallic cellular slime molds is regulated by pheromones. The species which have been shown to produce macrocyst-inducing pheromonal activity include *Dictyostelium discoideum*[3-5], *D. giganteum*[6], *D. mucoroides*[7-10], *D. purpureum*[11], and *Polysphondylium pallidum* (unpublished results). In contrast, *D. rosarium* apparently does not produce sex pheromones[12]. The accumulated data reveal that the sex pheromones of the cellular slime molds are volatile but none has yet been characterized. Ethylene can induce macrocyst formation in *D. mucoroides* and inhibitors of ethylene synthesis inhibit macrocyst formation suggesting that ethylene or a derivative may be the pheromone in this species[9]. A very tiny amoeboid cell which appears to represent the gamete phase of *D. discoideum* has recently been discovered[13,14]. These cells fuse producing binucleate and multinucleate cells which form the zygote of this species. Furthermore, these gametes are produced in much higher levels in the secreter (NC4) strain than the responder (V12) strain of this species[13]. This suggests a possible relationship between the pheromone-producing ability of a strain and its competence to produce gametes. The results presented here on early sexual development and on gamete production in single strains of *D. giganteum*, which comprise a unique mating hierarchy[6], supports this concept.

Materials and methods. Four mating type strains (WS588, WS589, WS606, WS607) of *D. giganteum* were maintained as stock fruiting body cultures on SM agar plates with *Escherichia coli* as a food source. For mixed mating type cultures spores of WS588 and WS589, which represent the opposite ends of the pheromonal hierarchy[6], were treated as detailed previously for

The cytoplasmic and nuclear volumes of cell types in sexual cultures of *Dictyostelium giganteum*

Cell type	Cytoplasmic volume (μm^3)	Nuclear volume (μm^3)
Amoebae	123.73 ± 3.94	13.80 ± 0.72
Gametes	43.16 ± 1.63	5.67 ± 0.16
Zygotes	450.88 ± 19.19	54.28 ± 2.47
Macrocysts	565.59 ± 29.51	NA

A mixed mating type culture (WS588 × WS589) was made as detailed in 'Materials and methods'. Cell aliquots were removed at specific times, placed on slides, fixed and stained with the nuclear fluorochrome Hoechst 33258. Photographs were made and the diameters of each cell type were measured with calipers coupled to a microcomputer which calculated the cell volumes[13]. The data represent the means and standard error as determined from measuring at least 24 cells of each type. NA, not applicable.