Table II. Analysis of variance of critical thermal maxima of chorus frogs

Source	SS	df	MS	F	P
Acclimation temperature	56.454	1	56.454	100.4	< 0.001
Elevation	24.067	1	24.067	42.8	< 0.001
Interaction (temperature × elevation)	1.803	1	1.803	3.2	> 0.1
Among populations (pooled)	4.499	8	0.562	43.2	< 0.001
Error	0.632	48	0.013		

Since there was significant variation in CTM among the populations of an experimental group, the Mean Squares/Among Populations was used as the denominator in calculating F-ratios for higher levels of variation[14]. SS, sums of squares; df, degrees of freedom; MS, mean squares; F, F-ratio.

determination of CTM was approximately 10 days for montane frogs and 30 days for piedmont animals.

Critical thermal maxima were subjected to 2-way analysis of variance, the fixed factors being elevation and acclimation temperature. One level of nesting (populations) was incorporated into the experimental design to permit us to distinguish altitudinal effects from interpopulation effects[14].

The CTM of chorus frogs acclimated at 20 °C was about 2 °C higher than that of frogs held at 5 °C (Tables I and II), which is the expected pattern of response[5, 13, 15]. More important, however, the CTM of piedmont frogs averaged 1.2 °C higher than that of montane animals (Tables I and II). Additionally, the effect of thermal acclimation on CTM was similar in frogs from montane and piedmont populations (Table II).

The CTM of amphibians has been reported either to be positively correlated with body weight[16] or to be independent of body weight[5–7]. Assuming that the former situation obtains in chorus frogs, it is possible that the CTM of the larger montane frogs[17, 18] was displaced upward relative to that of the smaller piedmont animals. However, since the observed CTM of the montane frogs still is lower than that of piedmont frogs (Table I), the difference in mean values seems not to be attributable to the influence of body weight.

Additionally, the CTM of amphibians has been reported either to decrease in starved animals[16] or to be independent of nutritional state[7]. Since piedmont frogs were held without food for a longer interval before study than montane frogs, it is possible that the CTM of piedmont animals was depressed accordingly. However, since the recorded CTM of piedmont frogs still is higher than that of montane frogs, the mean difference in CTM seems not to be attributable to different nutritional states of the experimental animals.

We conclude, therefore, that the different CTMs of montane and piedmont chorus frogs represent ecotypic variation within this species. The observed difference in CTM between montane and piedmont chorus frogs is precisely what one would predict as an outcome of evolutionary compensation for temperature[1]. The montane frogs, which occupy generally cooler habitats[10], presumably have acquired rate limiting enzymes in intermediary metabolism that function optimally at lower mean temperatures than do equivalent enzymes in piedmont frogs. Consequently, negative modulation of enzyme function by increasing temperature leads to metabolic inactivation, and death, at lower body temperatures in montane frogs than in piedmont animals[1].

Zusammenfassung. Das kritische thermale Maximum von Fröschen (Hylidae: *Pseudacris triseriata*) aus Bergpopulationen liegt niedriger als jenes von Tieflandpopulationen, wobei es sich offenbar um das Ergebnis einer physiologischen Evolution handelt: Adaptation an kühleres Höhenklima.

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Nicotinic Acid Suppressors in *Aspergillus nidulans*

Suppressor genes have been studied both in higher organisms and micro-organisms; and mainly in the latter, the study has led to important contributions to the understanding of gene action. In fungi, several instances have been reported of differences in dominance of suppressors between diploids and heterokaryons[1–3]. In respect to the nicotinic acid requirement caused by the *nic 8* mutant of *A. nidulans*, several authors reported the presence of suppressors of such gene. WARR and ROPER[4], for instance, looking for resistant mutants to various inhibitors, found that *p*-fluorophenylalanine (*p*FA) resistant genes also suppress the nicotinic acid requirement caused by *nic 8*.

We have isolated and studied suppressors of the *nic 8* gene of *A. nidulans* in relation to several aspects, such as map location, resistance to *p*FA, growth rate and dominance or recessiveness of the isolated suppressors in heterokaryons and diploids. For that the minimal medium

Table I. Ratio of segregant spores from crosses involving *su nic8*, *nic8* and their respective wild alleles

Crosses		Segregants		X^2 [a]
Suppressor involved	Tester strain	*nic*+	*nic*−	
su1	× bi1; *ni103*	304	112	0.83
su2	× bi1; *ni103*	90	14	7.39[b]
su3	× pro1, *paba6*; *v10*	83	21	1.28
su4	× pro1, *paba6*; *v10*	90	14	7.39[b]
su5	× bi1; *ni103*	154	54	0.10
su6	× pro1, *paba6*; *v10*	103	1	32,05[c]
su7	× pro1, *paba6*; *v10*	103	43	1.54
su8	× pro1, *paba6*; *v10*	166	42	2.56
su9	× pro1, *paba6*; *v10*	124	22	7.68[b]

[a] To fit a 3:1 segregation; [b] Significative at 1% level; [c] Significative at 1‰ level.

Table II. Growth rate of suppressors measured on a medium lacking nicotinic acid compared with growth of a *nic*+ strain

Strain	Efficiency (%) [a]
nic+	100,0
su1	84.5[b]
su2	101.2
su3	71.4[b]
su4	69.0[b]
su5	80.0[b]
su6	103.6
su7	29.8
su8	100.0
su9	103.6
nic−	0.0

[a] Taken the growth of *nic*+ strain after 96 h incubation as 100%
[b] Significative at 1% level.

(MM) was Czapek-Dox medium with 1% (w/v) glucose, Complete medium (CM) was a complex medium containing yeast extract, hydrolysed casein, hydrolysed nucleic acids, vitamins, etc[5]. Solid media contained 2% agar. The strains used were: strain MSE: *sul ad20, y, ad20; w3; gal1; pyro4; facA 303; s3;nic8;ribo2;* strain *bil; ni103;* strain *pro1, paba6; v29;* strain *sul ad20, y, ad20; phen2; pyro4; lys5; s3; nic8; ni103* and strain *pro1, paba6; v10.* Mutant alleles in this study determined the phenotypes: *y,* yellow conidia; *w3* (epistatic to *y*/*y*+), white conidia, *v10* and *v29,* brown mycelium; *ad20, bi1, lys5, ni103, nic8, paba6, phen2, pro1, pyro4, ribo2* and *s3,* requirements respectively for adenine, biotin, lysine, nitrites, nicotinic acid, *p*-aminobenzoic acid, phenylalaninine, proline, pyridoxine, riboflavine and thiosulfate; *sul ad20,* suppressor of adenine requirement caused by *ad20; facA 303* and *gal1,* inability to grow on medium with sodium acetate and galactose as the only source of carbon respectively. Origin of the strains and location of the mutants can be found elsewere[6–9]. General techniques of genetic analysis were those of PONTECORVO et al.[5]. The suppressor genes were obtained by UV-induced mutation by irradiating a suspension of conidia (MSE strain) with an UV-light source (2537 Å) to give 5% survival. 9 independent isolates (designed *su1* to *su9*) were then crossed with strains *bi1; ni103* and *pro1, paba6; v10* and mitotic and meiotic analysis were performed in the usual way[10–12]. The allelism relationships among the suppressors were studied by crossing two by two, different isolated suppressors and analysing ascospores from the hybrid perithecia obtained. The dominance: recessiveness relationship was studied in heterokaryons and diploids homozygous for *nic8* and heterozygous for the suppressor. Resistance to *p*FA was investigated on CM plus several concentrations of *p*FA added to the medium. The growth rate of strains with the different suppressors was investigated on MM lacking nicotinic acid.

Results and discussion. All 9 independent nicotinic acid isolates gave in crosses with nicotinic acid independent strains (*nic*+ strains) *nic*− segregants indicating the presence of suppressors (Table I). In 5 cases the suppressor was not linked to *nic8* gene since a 3 *nic*+: 1 *nic*− proportion of segregants was obtained. In 3 cases a loose linkage seems to be present and in the last case a strong linkage was observed.

Results from the mitotic and meiotic analysis have shown that all 9 suppressors were located in linkage group VII. (Figure). Test of allelism confirmed these results; MSE su *nic8* strains were crossed with *pro1, paba6, v29* strain and segregants containing the *nic8* allele and its suppressor were crossed to other strains carrying suppressors independently isolated; crosses between *su1*×*su3, su2*×*su9, su4*×*su2* and *su9*×*su4* produced no *nic*− segregants. In all other crosses *nic*− segregants were obtained. The results led to the conclusion that *su1* is allele of *su3* and that *su2, su4* and *su9* are

Table III. Resistance level of suppressors of *nic8* allele to *p*-fluorophenylalanine on CM

Strain	*p*FA (µg/ml)
MSE	< 250
su1	250–500
su2	250–500
su3	>1000
su4	>1000
su5	500–1000
su6	250–500
su7	< 250
su8	>1000
su9	500–1000

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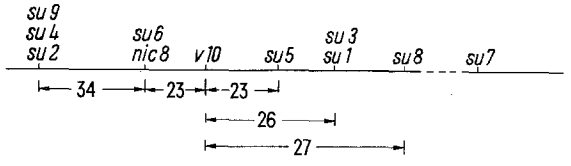
also alleles. Crosses involving *su7* deserve further consideration. In all crosses involving such strain, 'crinkled' colonies were obtained. Similar colonies were obtained by other authors in *A. nidulans*[13,14], and in all cases a duplication was involved. In *su7* case all crinkled colonies were phenotypically *nic*$^+$; it is possible that a duplication is involved which causes the crinkled phenotype and independence to nicotinic acid.

The dominance-recessiveness relationships among the 9 suppressors were carried out by formation of heterokaryons homozygous for *nic8* allele and heterozygous for the suppressor tested (MSE *su nic8* × *su1 ad20, y, ad20; phen2; pyro2; lys5; s3; nic8; ribo2*) and by growing them on MM supplemented with pyridoxine, thiosulfate and riboflavine. In all 9 cases the heterokaryon could grow on this medium. On the other hand, with one exception, diploids obtained from such crosses were unable to grow on the same medium. The exception was the diploid heterozygous for *su6*, probably an intragenic suppressor. These results were taken as experimental support for the hypothesis about the mechanism of gene regulation, which is an adaptation to the 'Cascade regulation mechanism' proposed by PONTECORVO[1]. According to this hypothesis, *nic8* would be a mutant in the regulator R_2 which does not make a repressor active to block the action of the regulator R_1, thus inhibiting the action of the structural gene. In our case, the other loci would be collectively responsible for the syntheiss of the other repressor. Mutation in one or more of these loci would be sufficient to prevent the synthesis of the second repressor, thus enabling the structural gene to synthesise its enzyme. Such repressor (R_1) would be made of several units resulting in a complex chain. Each of these units would be produced by a specific locus, where any mutation results in an absent or altered subunit and, consequently in a non-functional complex. Of course, other explanations could be proposed, as, for instance, an adjustment on MM in favour of 1 nucleus in heterokaryons[3]. On the other hand, the nuclear location of repressors cannot be ruled out and the existence of systems of regulation at nuclear level was already proposed for eucaryotes[15]. All suppressors analysed appear to exhibit striking differences in their gene structures, since they showed difference in growth rates (Table II). Also they exhibit a pleiotropic effect, since most of them conferred at the same time independence to nicotinic acid requirement and resistance to *p*FA (Table III). Such effects can be explained by the fact that some substances including shikimic acid are common precursors of phenylalanine and nicotinic acid[4]. More consistent knowledge of the suppressor genes used by us could however, only be obtained from more detailed genetic and biochemical studies.



Location of the different suppressors of *nic8* allele analysed (linkage group VII). Distance are only approximated and are not in scale on the map.

Zusammenfassung. Neun Suppressoren des Mutanten *nic8* von *Aspergillus nidulans* wurden in der Kopplungsgruppe VII der Chromosomenkarte lokalisiert und unter verschiedenen Aspekten studiert. Eine Hypothese über die Mechanik der genetischen Regulierung der Erzeugung der Nikotinsäure wurde formuliert.

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Effect of Influenza Virus PR8 Infection on Thymus in Intact and Adrenalectomized Mice

The mechanisms of thymus involution are very complex and not well understood. Many external agents responsible for thymus involution instead of acting separately trigger intrinsic mechanisms of the organism, such as the secretion of corticosteroids, which contribute greatly to thymolysis (for ref. see [1]). This laboratory has previously reported[2] a drastic reduction in thymus weight after influenza virus PR8 infection of mice. In the present study we analyzed the extent of thymus reduction caused by virus infection in adrenalectomized mice as compared with thymus damage in intact infected mice. We were also looking for morphological differences in intact and adrenalectomized mice.

Materials and methods. Highly inbred BALB/c mice, 6 weeks old, intact, adrenalectomized[3] and sham adrenalectomized (10 animals per group) were inoculated intranasally with 0.1 ml of influenza virus suspension (PR8, A_0 type, 13 H.A.U./ml). Viral infection was controlled by the presence of serum antibodies detected by haemagglutination inhibition test. Adrenalectomized mice showed higher susceptibility to the virus, but the infection was only rarely fatal. Thymuses of all groups of mice were collected daily, from 1st to 10th day after virus inoculation, after fixation in situ (DJACZENKO and CIMMINO[4]). Tissue was embedded in Vestopal W and semithin sections were stained with 0.1% toluidine blue.

Thymuses presented in Figures 2–5 were prefixed in situ with a mixture of acrolein-glutaraldehyde and TAPO[4], postfixed with 4% osmium tetroxide, dehydrated and embedded in Vestopal W. Semi-thin sections (0.5 μm thick) were stained overnight with 0.1% solution of toluidine blue in 1% borax.

Results. Figure 1 shows that thymuses of infected mice undergo a drastic reduction of weight, more pronounced in intact animals. Morphology of the thymus of normal control mice may be observed in Figure 2. Thymuses of

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