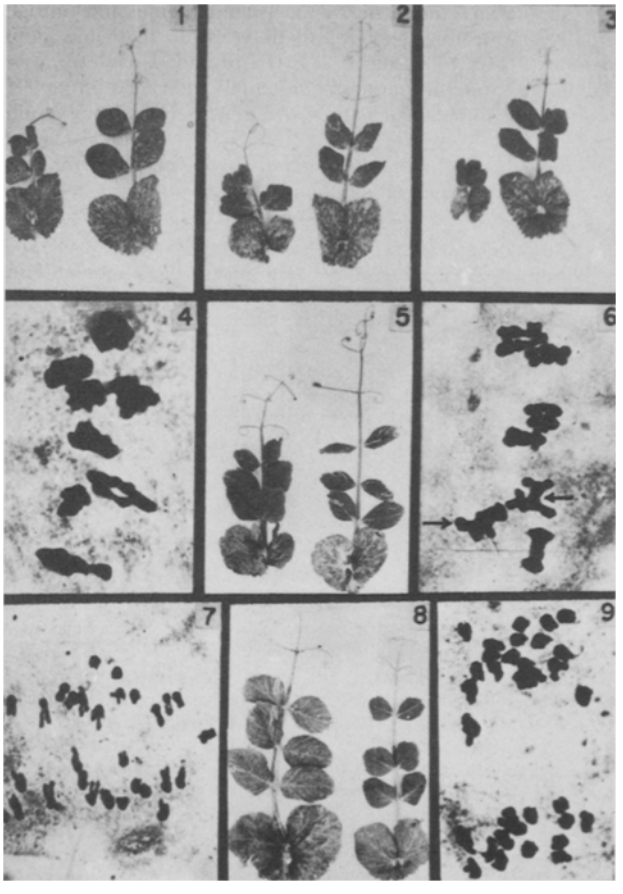

Figures 1–3. Leaves of three hexasomic tetraploids (left) and corresponding controls (right) of pea. Figure 1. L282-1. Figure 2. L81-4. Figure 3. L157-3. Figure 4. Metaphase I of hexasomic tetraploid showing 7IV+1II (× 1000). Figures 5, 6. Leaf and metaphase I of double pentasomic tetraploid of pea. Figure 5. L136-3 (left) and control (right). Figure 6. Metaphase I, 2V+5IV (pentavalents arrowed) (× 1000). Figure 7. Anaphase I, 15 and 15 disjunction (× 600). Figures 8, 9. Leaf and anaphase I of multiple aneuploid tetraploid in pea. Figure 8. L266-15 (left) and control (right) (× 600). Figure 9. Anaphase I, 19 and 14 disjunction (× 800).

Internodal length, total leaf length and tendrils were highly reduced. Leaflets were clustered. Stipule and leaflets were small in size.

The double pentasomic tetraploid (L136-3, 4n+1+1 = 30) was a dwarf with a thick stem, highly reduced internode and poor vegetative growth. The round stipule and the leaflets were compactly arranged. The plant flowered earlier by 13 days.

The multiple aneuploid tetraploid (L266-15, 4n+2+1+1+1 = 33) was characterized by its thick stem, very vigorous growth with highly increased size of leaflets and stipules and late flowering by 30 days.

Cytologically the hexasomic tetraploids were characterized by the presence of a hexavalent (average ranged from 0.07 to 0.30 per cell) and a high frequency of cells with 7IV+1II. These hexasomic tetraploids did not show much variation with respect to chromosome configurations at metaphase I (MI).

Anaphase I (AI) abnormality was high, amounting to 80% in L81-4 and L157-3 as compared to about 50% in L282-1 and the euploids. While in the double pentasomic tetraploid about 10% of the cells showed 2V+5IV configuration at MI; AI abnormality was observed in about 70% of the cells. In the multiple aneuploid tetraploid, besides quadrivalents, trivalents, bivalents and univalents, cells with a hexavalent (0.50%) and pentavalents (2%) were also noted at MI, suggesting the presence of aneuploidy for different linkage groups. AT AI, 90 of the cells had abnormal separation. Pollen fertility in these hyperploid tetraploids ranged from 19 to 50%; remarkably, none of them produced any seed, which suggests a lack of correlation between pollen fertility, as judged from the stainability of pollen grains, and seed set.

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Eosinophilic granulocyte deficiency in mice mutant in *sl* and *w* loci

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Summary. Homozygous *Sl/Sl* and W^v/W^v mice were found to have approximately 15% of the normal number of circulating eosinophils. Furthermore, these mice exhibited reduced numbers of eosinophilic granulocytes in the bone marrow, spleen and thymus as compared to littermate control normal mice.
Key words. *Sl* mutation; *W* mutation; eosinophils.

The expression of the semidominant mutations of *sl* and *w* genes of mice result in a number of effects on hemopoiesis, germ cells and neural crest melanocytes[2]. Differences exist between these various mutant alleles with regard to the resulting abnormalities[2]. However, in all these mutants hemopoiesis is affected markedly[3]. Deficiences of mast cells[4,5], erythrocytes[3], neutrophils[6,7], megakaryocytes[7,8], and lymphocytes[9] have been described. However, no studies of eosinophilic granulocytes in these mice have been reported to date. Additional interest in the possible effects of *w* and *sl* loci on eosinophilic granulocytes arises from the possibility of the existence of an independent origin of this cell line from the stem cell compartment[10].

Materials and methods (WC × WC Swiss F_1) BC_1 mice were bred as previously described[11] and were of the following genotypes: +/+, *Sl*/+ and *Sl/Sl* in *sl* locus. W^v/W^v mice were bred similarly using the backcross method[11]. Briefly, C57Bl/6-W^v/+ mice were

Table 1. Eosinophilic granulocytes in various organs of steel mutant and littermate control mice

Parameter	Sex and genotype of mice – Number of cells ± SD[a]					
	Female			Male		
	+/+	Sl/+	Sl/Sl	+/+	Sl/+	Sl/Sl
Absolute blood eosinophil count × 10^9/l	0.63 ± 0.15	0.44 ± 0.44[b]	0.09 ± 0.04[c]	0.67 ± 0.34	0.47 ± 0.16[b]	0.11 ± 0.04[c]
Eosinophils per femur × 10^6	1.6 ± 0.7	1.4 ± 0.6	0.4 ± 0.2[c]	1.7 ± 0.6	1.5 ± 0.3	0.4 ± 0.2[c]
Eosinophils per humerus × 10^6	0.8 ± 0.3	0.7 ± 0.3	0.2 ± 0.2[c]	0.5 ± 0.2	0.5 ± 0.2	0.2 ± 0.1[c]
Eosinophils per spleen × 10^6	2.7 ± 1.6	1.9 ± 0.4	0.4 ± 0.1[c]	3.5 ± 1.7	1.8 ± 1.0	0.6 ± 0.3[c]
Eosinophils per thymus × 10^6	0.09 ± 0.07	0.08 ± 0.05	0.01 ± 0.001[c]	0.11 ± 0.08	0.09 ± 0.07	0.01 ± 0.01[c]

[a] Seven littermate triads of +/+, Sl/+, Sl/Sl mice of each sex were tested and data were evaluated using paired t-tests. [b] Significance level $p < 0.05$ when compared to +/+ mice. [c] Significance level $p < 0.001$ when conpared to +/+ control mice.

first mated to Swiss: NIH mice and the resulting (B6 × Swiss) F_1-W^v/+ mice were backcrossed to C57Bl/6-W^v/+ mice. Subsequently, (B6 × [B6 × SwissF_1]) BC_1 mice, which were +/+, W^v/W^v in the *w* locus, were tested. Both female and male mice aged between 3 and 28 weeks were evaluated, although the majority of the mice were 12 weeks old. Because of considerable variability in the results in preliminary experiments, the study on *Sl/Sl* mice was performed using triads of mice from the same litter and housed together. W^v/W^v mice, control and mutant animals, were matched for age, sex and genotype, but not necessarily from the same litter.

Mice were bled from the retroorbital sinus and then killed by cervical dislocation. Their peritoneal and pleural cavities were washed with 2 ml of Hanks Solution (HBSS, Biomed, Poland) and femurs, humeri, spleens and thymi were removed. Bone marrow cells were independently and quantitatively obtained from femurs and humeri by cutting both ends off the bones and washing the marrow cavities with defined volumes of HBSS, followed by careful washing of the ends of the bones to remove remaining cells. Spleens and thymi were individually teased using a rubber spatula in 5 ml of HBSS. Single cell suspensions of marrow, spleen and thymus cells were made by repeated aspiration with a needle and syringe.

Eosinophils in peripheral blood, the peritoneal and pleural lavages, and marrow, spleen and thymus cells were directly counted using Hinkleman's fluid[12] (eosin gelbl. 0.5 g, formal. conc. 0.5 ml, phenol 95% 0.5 ml, aqua dest. ad 100 ml) in a Fuchs-Rosenthal chamber. This fluid stains eosinophilic granules exclusively and, therefore, allows the enumeration of cells from the eosinophilic promyelocyte stage to segmented eosinophils. In preliminary studies, parallel counts of all cells belonging to the eosinophilic lineage from normal murine bone marrow using either Wright's stained preparations or Hinkleman's fluid yielded equivalent results.

Results were analyzed using either the paired t-test (*Sl/Sl* mouse data) or t-test (W^v/W^v mouse data).

Results and discussion. As shown in table 1, both female and male 12-wk-old *Sl/Sl* mice had a severe reduction in the number of eosinophils in all organs evaluated. Absolute eosinophil counts in the peripheral blood were decreased by 85%. The most significant reduction in number was observed in the spleen where the *Sl/Sl* mice had between 10 and 20% of the eosinophils normally present in this organ. A large number of eosinophils was found in the normal thymus and smaller numbers were observed in *Sl/Sl* mice thymi. Data for peritoneal and pleural lavages are not included in table 1 because very few eosinophils were found in these locations in normal mice; there were not enough to perform a quantitative analysis. *Sl*/+ mice usually had smaller numbers of eosinophils than +/+ mice; however, because of the large variability observed, only the differences seen in the peripheral blood of mice expressing the *sl* mutation were significant.

As shown in table 2, both female and male 12-wk-old W^v/W^v mice had reduced eosinophil counts in all organs evaluated compared with the +/+ mice. Again, the most marked reduction in numbers was in the peripheral blood and in the spleen, although in all organs the eosinophil counts of W^v/W^v mice were several times smaller than in either +/+ or W^v/+ mice. W^v/+ mice had consistently smaller eosinophil counts in various organs. However, because of large individual variability, these differences were not significant.

Neither in *sl* mutant mice nor in *w* mutant mice were any significant differences in eosinophilic granulocyte counts related to sex (tables 1 and 2) observed.

Both younger mice (up to 3 weeks of age) and older ones (up to 28 weeks of age) demonstrated similar differences in the eosinophil counts in the blood and other organs (data not shown).

In summary, this study has documented that *Sl/Sl* and W^v/W^v mice, in addition to expressing other defects[3], possess a marked deficiency in total numbers of eosinophils at all morphologically identifiable levels of maturation. In comparison to the other defects in hemopoietic differentiation[3], with the exception of the mast cells, the defects in the eosinophilic system are more severe. Both *Sl/Sl* and W^v/W^v mice demonstrated eosinopenia of similar severity. This result is in contrast to the degree of the anemia present in these mice since *Sl/Sl* mice have hematocrit levels of 0.33 on the average[11], while W^v/W^v mice have 0.41 on the average (unpublished observation).

To our knowledge, mice possessing the mutant alleles in *sl* and *w* loci are the only animals with demonstrated inherited eosinophil deficiency. Therefore, these mice should be useful for studies of eosinophil differentiation, maturation and function.

Table 2. Eosinophilic granulocytes in various organs of *w* mutant and control mice

Parameter	Sex and genotype of mice – Number of cells ± SD[a]					
	Female			Male		
	+/+	W^v/+	W^v/W^v	+/+	W^v/+	W^v/W^v
Absolute blood eosinophil count × 10^9/l	0.59 ± 0.16	0.47 ± 0.19	0.08 ± 0.01[b]	0.56 ± 0.14	0.39 ± 0.15	0.09 ± 0.04[b]
Eosinophils per femur × 10^6	1.9 ± 0.3	1.8 ± 0.4	0.4 ± 0.1[b]	1.8 ± 0.3	1.5 ± 0.5	0.5 ± 0.1[b]
Eosinophils per humerus × 10^6	0.8 ± 0.2	0.7 ± 0.1	0.2 ± 0.1[b]	0.7 ± 0.2	0.4 ± 0.3	0.2 ± 0.9[b]
Eosinophils per spleen × 10^6	3.3 ± 1.2	2.3 ± 0.7	0.3 ± 0.2[b]	2.8 ± 0.6	2.5 ± 0.5	0.6 ± 0.2[b]
Eosinophils per thymus × 10^6	0.12 ± 0.06	0.08 ± 0.04	0.03 ± 0.01[b]	0.12 ± 0.05	0.08 ± 0.01	0.03 ± 0.01[b]

[a] Four mice per group. Data were analyzed using t-tests. [b] Significance level of $p < 0.001$ when compared to +/+ control mice.

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Abnormal development of phialides in a strain of *Aspergillus niger*[1]

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Summary. An *Aspergillus niger* mutant strain *(hpp)* produces an average of 4.1% of conidiophores with phialide proliferations. Increased frequency of proliferations could be induced on all studied strains by growth on potato dextrose agar. The characteristic is recessive and seems to be due to a pleiotropic effect of the mutation for olive conidia color.
Key words. Aspergilus niger; conidiophores; phialide proliferation.

Aspergillus niger strains are used in the industrial production of citric acid, as well as others metabolites of industrial interests[2,3]. These strains and others isolated from nature generally have a normal development of their asexual reproducing structures, but in some adverse conditions, phialides proliferate into conidiophores. These conditions are: growth in malt agar[4], nearly anaerobic conditions[5] or transfer from low nitrogen media to nitrogen containing media with citrate as a carbon source[6]. Other authors[7-9] observed proliferations in others species of *Aspergillus* and in *A. proliferans;* these proliferations are considered the most conspicuous species characteristic[10]. In this paper we will describe the isolation of a mutant from an industrial strain of *A. niger* with a high frequency of phialide proliferations, and the influence of culture media and the genetic study of this characteristic.

Materials and methods. The parental strain, HCA, was identified as *Aspergillus niger,* according to Raper and Fennel[10], and mutants were obtained by Bonatelli Jr et al.[11]. The minimal medium (MM) used was Czapek-Dox with 1% of glucose. The complete medium (CM) contains all the substances of MM plus yeast extract, hydrolyzed casein, peptone, hydrolyzed nucleic acid and vitamins[12]. Potato Dextrose Agar (PDA) was prepared with 200 g of peeled potatoes, 10 g of dextrose and 15 g of agar in 1 l of distilled water. The vitamins and adenine were added to MM and PDA in final concentrations of 5 µg/ml and 50 µg/ml, respectively. The number of proliferations was recorded by counting 400–800 conidiophores after 7 days of incubation at 28 °C. In some cases, the presence or absence of proliferations was observed with a stereomicroscope (× 40) in plates containing MM, CM or PDA. In these cases, the number of conidiophores with proliferations was not recorded. The general genetic techniques were those of Pontecorvo et al.[12] and Lhoas[13]. Heterokaryons were formed in liquid MM with 4% (v/v) of CM. Diploids $pur_1//pdx_1olv_1$ and $pab_1fwn_1//pdx_1olv_1$ were isolated by the technique of Roper[14]. The segregants were isolated by inoculating conidia of the diploid strains on solid CM containing 1–3 µg/ml of Benlate[15]. The ploidy of the strains was determined by measuring the conidial diameter[13].

Results and discussion. The presence of proliferations was observed when the strain pdx_1olv_1 was analyzed cytologically, because it has no morphological alteration with the naked eye. Consequently this strain was denominated as *hpp* (high phialide proliferations). Later, it was observed that proliferations were visible under a stereomicroscope and this fact permitted the screening of several colonies with little work. On MM the frequency of proliferations of *hpp* strain is 4.7–16.4 times greater

Table 1. Effect of culture media on the frequency of proliferations in *Aspergillus niger* strains

Culture media	Strains	Total number of conidiophores scored	Number of observed proliferations/ conidiophore	Conidiophores with proliferations (%)	Conidiophores with one proliferation (%)
PDA*	HCA	400	1–2	15.0	14.5
	pab_1fwn_1	800	1–3	23.4	22.8
	pdx_1olv_1	800	1–4	41.4	33.1
	$pdx_1olv_1//pab_1fwn_1$	656	1–3	23.8	21.8
MM*	HCA	400	1	0.3	0.3
	$pdx_1olv_1//pab_1fwn_1$	800	1	0.6	0.6
MM* supplemented with					
pab**	pab_1fwn_1	800	1	0.9	0.9
pdx**	pdx_1olv_1	800	1	4.1	4.1

*PDA: potato dextrose agar; MM: minimal medium. ** pab: *p*-aminobenzoic acid; pdx: pyridoxine.