Reversion of virus N-1 resistant mutant of blue-green alga *Nostoc muscorum*

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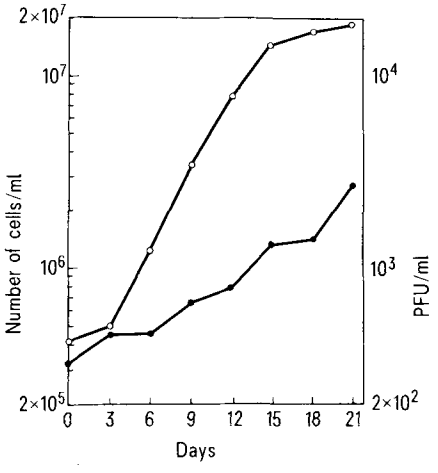
Summary. The reversion of N-1 virus resistant strain of the alga *Nostoc muscorum* was studied by inoculating parent virus in the resistant culture at various incubations. A fraction of virus resistant cells reverted to wild sensitive type with the reversion rate of 3.99×10^{-6}/cell/generation.

The spontaneous and induced mutation in the blue-green algae *Plectonema boryanum* and *Nostoc muscorum* resistant to LPP-1 and N-1 respectively is established[2-4]. However, there is no previous report on the reversion of virus-resistance in blue-green algae. The present work deals with the reversion of N-1 virus-resistant cells of *N. muscorum.*

The nitrogen-fixing blue-green alga *Nostoc muscorum* and virus N-1 were used in the study. Aliquots of 0.5 ml of a clonal population of a virus resistant isolate was inoculated into a number of tubes containing 9.5 ml of growth medium. These were divided into 2 series. The 1st series was kept under light in a culture-room, for cell counting at intervals of 3 days after 1:10-fold dilution. To the 2nd series, aliquots of 0.1 ml of virus suspension (3.2×10^5 plaque forming units/ml) were added and these tubes were incubated under light along with the 1st series; the virus titre of this series was determined at intervals of 3 days after serial dilutions. The back-mutation rate (m_b) of virus-resistance was calculated by the formula: $m_b = \log_e 2(M_2 - M_1)/(N_2 - N_1)$[5], where M_1 and M_2 are numbers of virus sensitive cells (calculated from the increase in virus titres and the average burst-size) arising at times 1 and 2 and N_1 and N_2 are the corresponding total cell counts.



Growth of virus resistant isolate (○) and virus-titre (●) in resistant culture after virus addition.

The cell number of virus-resistant strain and virus titre in the supernatant of the culture after addition of virus are illustrated in the figure, from which the back-mutation rate of virus-resistant cells to virus sensitivity was calculated. The increase in virus titres between 3rd and 15th day of incubation was 7.5×10^3 PFU/ml. Since the average burst-size of this virus in parent[6] and revertant is 120 PFU/ml, the increase in virus titre (7.5×10^3 PFU/ml) was equivalent to 62.5 cells/ml, whereas the increase in total cell counts within this period was 10.9×10^6 cells/ml. Thus, the mutation rate of virus-resistant population was calculated to be 3.99×10^{-6}/cell/generation.

1 We gratefully acknowledge Dr H.K. Pande and Dr S. Patnaik, Crops and Soils Division.
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Environmental and racial influences on the masticatory apparatus of mediterranean and atlantic populations of *Ophryotrocha labronica* (Annelida, Polychaeta)

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Summary. Significant differences in the length of the definitive upper jaw have been ascertained in 3 populations of the comprehensive species *O. labronica.* The ratio of the variance (F) of the 3 populations has been employed in evaluating the taxonomic distance between the populations. The density of the water and the kind of food strongly influence the length of the definitive upper jaw.

La Greca and Bacci[1] described *Ophryotrocha labronica* on a proterandric hermaphrodite population from Livorno. Åkesson[2] later examined a number of *O. labronica* from different seas, and established that all of them were gonochoric with the exception of a population from Faro (Portugal) which was partly hermaphroditic. Åkesson also found that sex ratios differ in the different populations from 28% males in Malaga to nearly 50% in Famagusta (Cyprus) and Palma de Mallorca. Varying degrees of reproductive isolation were also ascertained between populations ranging from complete isolation to alteration in the sex ratios of hybrids.

The hard pieces of the complicated definitive masticatory apparatus[3,4] proved to be very useful in identifying the numerous species of *Ophryotrocha.* Measurements of such pieces are employed in the present research to check whether environmental factors have any influences on the size of the definitive upper jaw, and whether significant biometrical differences can be demonstrated between populations pertaining to the comprehensive species *O. labronica.*

The investigation was carried out on adult individuals measuring 13 chaetigerous segments, which came from strains originally collected in the lagoons of Venice (VE), in

Naples (NA IV) and in Faro, Portugal (FA). Specimens of the strain from Faro were kindly provided by Prof. Åkesson. The Naples and Faro strains were kept in marine water of normal density, while the Venice strain was maintained in slightly brackish water. The temperature was maintained at about 18 °C and frozen spinach was given as food.

The length of the definitive upper jaw (DUJ) can be measured with accuracy on preparations which were obtained from squashes of the whole masticatory apparatus of individuals from the different strains which were previously sexed.

The influence of water density on the length of the DUJ was demonstrated by maintaining the VE strain in water at the density of 1.025 (the density of the original samples) and at the density of 1.028.

The difference is highly significant with the DUJ of the males measuring 27.24±0.71 µm in the brackish water samples, 24.98±0.72 µm in the samples kept in ordinary sea water. The corresponding data concerning females are 16.17±0.42 µm and 14.06±0.63 µm respectively.

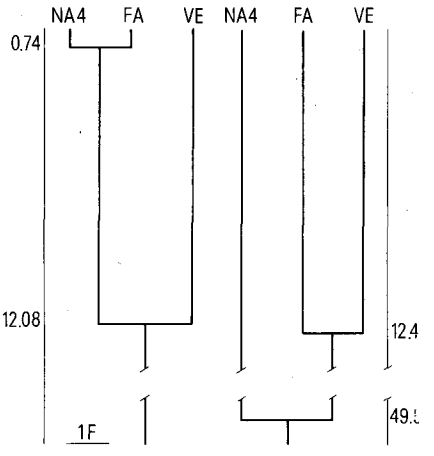
Table 1

Populations	DUJ of ♂♂	DUJ of ♀♀
a) Population in natural environment fed with spinach leaves ($\bar{x} \pm S_M$ in µm)		
Venezia	27.24±0.71	16.17±0.42
Napoli IV	24.98±0.49	12.98±0.29
Faro	25.42±0.52	14.72±0.38
b) Population in stronger density sea water, fed with spinach leaves		
Venezia	24.98±0.72	14.06±0.63
c) Population in stronger density sea water fed with *Dunaliella*		
Venezia	21.28±0.57	11 ±0.31

The analysis of the variance relative to the results summarized in table 1 are found in table 2.

The values of F obtained from the comparison between the 3 populations have been considered as suitable to express the taxonomic distances between the populations, and the following dendrograms have been obtained on the basis of such values which were elaborated according to the UPGMA method[5]: least dissimilar values, which turned out to be FA, NA IV for males were clustered. Next value (FA+NA IV, VE) was computed simply by averaging FA, VE and NA IV, VE. The same procedure was employed for females, giving the other dendrogram.

It is must be remarked that the values of F relative to comparisons between the DUJ in the males of the 3 populations are low or not significant, while they are much higher and always significant in the females.



UPGMA dendrogram derived from F values of table 2. At left are males, at right females.

Table 2

Populations	$F_{0.05}$ d.f.$_{(1.98)}$ ♂♂		$F_{0.05}$ d.f.$_{(1.98)}$ ♀♀	
a) Comparisons between populations in natural environment fed with spinach leaves				
VE×NA IV	6.71	Significant	38.06	Significant
VE×FA	5.37	Significant	6.23	Significant
FA×NA IV	0.37	Not significant	11.25	Significant
Populations	$F_{0.05}$ d.f.$_{(1.38)}$ ♂♂		$F_{0.05}$ d.f.$_{(1.38)}$ ♀♀	
b) Comparison between populations in different density sea waters fed with spinach leaves				
VE×VE	2.77	Not significant	5.6	Significant
c) Comparison between populations in equal density sea waters fed with different food				
VE×VE	15.83	Significant	18.16	Significant

F, ratio of the variances of the 2 populations; d.f., degrees of freedom.

VE strains were also kept in sea water at the density of 1.028, but 1 sample was fed with spinach, another was fed with the alga *Dunaliella*.

Significant differences were also ascertained as a result of the 2 treatments with the DUJ measuring 21.28±0.57 µm in the males and 11±0.31 µm in the females fed with *Dunaliella* and measuring 27.24±0.71 µm and 16.17±0.42 µm in males and females fed with spinach.

It is thus demonstrated that environmental influences, such as water density and nutrition, can influence considerably the length of the DUJ. A remarkable degree of sex dimorphism is also demonstrated in the results of all the previous and the following observations thus confirming previous results by Åkesson[2].

The results of the experiments on the influences of water density and on the kind of food are summarized in table 1.

While, according to the results in the male individuals, the FA and NA IV populations appear to be distant from the VE population and rather close together, the results obtained in the female individuals strongly indicate a marked distance of the NA IV population from both FA and VE strains. The above results are being confirmed by a parallel research carried on with electrophoresis of the total proteins on the same populations[6].

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