Comments on the genetic stability of aphid clones[1]

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Summary. No segregants were found amongst 2343 descendants from 35 different clones of the aphid *Macrosiphum rosae* (L.). The clones were heterozygous with respect to the enzymes malate dehydrogenase and/or phosphoglucomutase.

The genetic stability of aphid clones is an extremely controversial area of discussion. Many aphid species undergo a seasonally induced change in their reproductive system. In general a sexual phase is induced in the autumn and the sexual morphs lay winter eggs from which, in the following spring, parthenogenetically propagating summer populations emerge. Aphids are viviparous and within an as yet unborn larva another individual is already developing. Owing to this fact, and also because of the smallness of the chromosomes, cytological investigations are extremely difficult. Therefore no agreement exists with regard to the type of parthenogenesis.

Whereas White[2] and Suomalainen et al.[3] believed in an apomictic parthenogenesis which permits no genotypic change in an aphid clone, other than by mutation, Cognetti[4] and Pagliai[5] called the observed meiosis an endomeiosis, which renders gene recombination possible. The statements of Cognetti and Pagliai are based upon cytological observations of *Macrosiphum rosae* (L.), Brevycoryne brassicae (L.), *Myzus persicae* Sulzer and *Acyrthosiphon pisum* Harris. Apart from these cytological investigations indirect experiments have been carried out, which either confirm or question the stability of aphid clones.

Müller[6] found in red clones of *Acyrthosiphon pisum* green coloured descendants and concluded that there had been segregation of alleles determining body color. Beranek and Berry[7] observed a change in the esterase patterns prevalent in clones of *Aphis fabae* Scopoli. In view the high frequency of newly observed types (2.3×10^{-3} and 6.25×10^{-3}), the authors excluded mutations and attributed the new types to recombinational events. Beranek[8] found a confirmation of this affirmation in experiments in which 3 out of 54 clones of *Myzus persicae* lost their resistance to insecticides during parthenogenetical propagation. There exists a disparity between these results and the investigations carried out by Blackman[9] and Suomalainen et al.[10]. Without any success, Blackman selected for normal and altered caudal hairs in 2 lines of *Acyrthosiphon pisum,* and for winged morphs in 6 clones of *Myzus persicae* up to 170 generations. Furthermore, Blackman investigated, electrophoretically, 5 clones of *Myzus persicae* heterozygous in 2 esterase loci. Once more, no segregants could be observed. Reaching the same conclusion, Suomalainen et al. analyzed, in total, 30 descendants of 2 clones of *Acyrthosiphon pisum* for malate dehydrogenase.

The question emerges whether these results confirm the concept of the genetical stability of aphid clones, and refute the idea of e.g. Cognetti. Assuming that new types occur with a certain frequency (r), the probability (P(r)) of finding at least one segregant in a random sample of size N is $P(r) = 1-(1-r)^N$. Considering the highest rate reported by Beranek and Berry ($r = 0.0063$), the probability that Blackman should find 1 segregant in a sample of 107 or 104 individuals stands at 0.49 and 0.48, respectively (table). In all other cases the probability is much less and, therefore, the results are not sufficient to refute completely the results of Beranek and Berry and consequently the existence of endomeiosis. All the more so if lower rates, e.g. $r = 0.001$, are taken into account. Furthermore, the results are based on a small number of clones; a fact which may be of importance if special chromosome structures require consideration. In order to reach a conclusive decision, a larger sample is required, i.e. a greater number of clones and loci. However, the fulfilment of this requirement causes some difficulties, because of the relatively low degree of polymorphism to be found in natural populations of aphids[9-11]. *Macrosiphum rosae* is polymorphic in MDH and PGM as well as in EST and SDH[14]. Despite the fact that it has not been possible up to now to prove the inheritance of the observed enzyme patterns due to the impossibility of controlled mating experiments, a genetic interpretation of the observed patterns can be given, at least in the case of MDH and PGM in analogy to the genetics in *Drosophila*[12]MDH is a dimer and 2-allelic yielding 3 distinct phenotypes and with regard to the PGM locus, 3 alleles are known, the result being 6 phenotypes. Previous observations show no indication of a linkage between the 2 loci.

The results obtained are based upon investigations carried out on clones of 31 double (MDH, PGM) and 4 single (MDH) heterozygotes, from which in total 2166 and 177 parthenogenetic descendants, respectively, were tested electrophoretically. The heterozygotes were collected from 12 different localities and propagated under summer conditions in an air-conditioned cabinet. Keeping in mind the rate of segregants found by Beranek and Berry[7] the probability of finding one in this particular sample is approximately 1.0. Even in the case of a very small rate of 0.001, the probability of finding a segregant is still 0.9 or 0.89. However, no segregant could be observed. These results support, but do not establish definitive proof of, the absence of recombination events during the parthenogenetic propagation phase of aphids.

Sample sizes (N) and the probabilities (P(r)) of detecting at least one enzyme segregant where r is the frequency of segregants

Author	Species	Enzyme	N	p(0.0063)	p(0.0023)	p(0.001)
Beranek and	*A. fabae*	EST	1750	–	–	0.83
Berry[7]	*A. fabae*	EST	800	–	–	0.55
Blackman[9]	*A. pisum*	EST-1	107	0.49	0.22	0.10
	A. pisum	EST-4	104	0.48	0.21	0.10
Suomalainen	*A. pisum*	MDH	30	0.17	0.07	0.03
et al.[10]	*A. pisum*	MDH	60	0.32	0.13	0.06
Tomiuk and	*M. rosae*	MDH	2343	~1.00	~1.00	0.90
Wöhrmann[12]	*M. rosae*	PGM	2166	~1.00	0.99	0.89

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Genetic control and modifications of peroxidase pattern in two species of *Nicotiana* and their interspecific hybrid

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Summary. The peroxidase patterns of *Nicotiana suaveolens, N. glutinosa* and their interspecific hybrid have been studied, and their possible genetic control analyzed. Modifications of the isozymatic pattern along the plant have been observed.

The peroxidases represent a wide group of isozymes present in plants generally, showing inter and intraspecific variability. This variability is also shown during plant development[2]. Almost all the genetic studies carried out with peroxidases coincide in showing their monomeric character and monogenic control[3–8] and in showing the existence of null alleles[3,8]. The only exception observed is in rice[9], in which a dimeric behaviour might be indicated.

In the present work, the study of the patterns of the leaf peroxidase isozymes of *Nicotiana suaveolens* × *N. glutinosa* hybrids and both parental species has been carried out, in order to analyse their genetic control, and also to detect the possible variation of the pattern along the plant.

Material and methods. Flowering plants of *Nicotiana suaveolens* (su) and *N. glutinosa* (glu) as well as their interspecific hybrid (su × glu) were used. The seeds of the *Nicotiana* species were supplied by the 'Centro Tecnológico del Tabaco', Sevilla (Spain) (su) and the 'Hortus Botanicus Bergianus', Stockholm (Sweden) (glu). The analyses were carried out on crude extracts of leaves taken at different plant levels; level 1 – the basal rostette, level 2 – intermediate, level 3 – floral branches. Peroxidase isozymes were studied using Tris-citric acid 0.015 M, pH = 7.75 as the gel (12% starch) buffer and NaOH-boric acid 0.3 M, pH = 8.6 as the electrode buffer. The gels were stained using the method described by Shaw and Cohen[12].

Results and discussion. Anodal peroxidases (APX) as well as cathodal peroxidases (CPX) were observed. In level 1 (basal rosette) the APX pattern showed a total of 6 bands (Nos 6, 7, 8, 9, 10 and 11) for *glu* and *su* × *glu*, and 5 bands

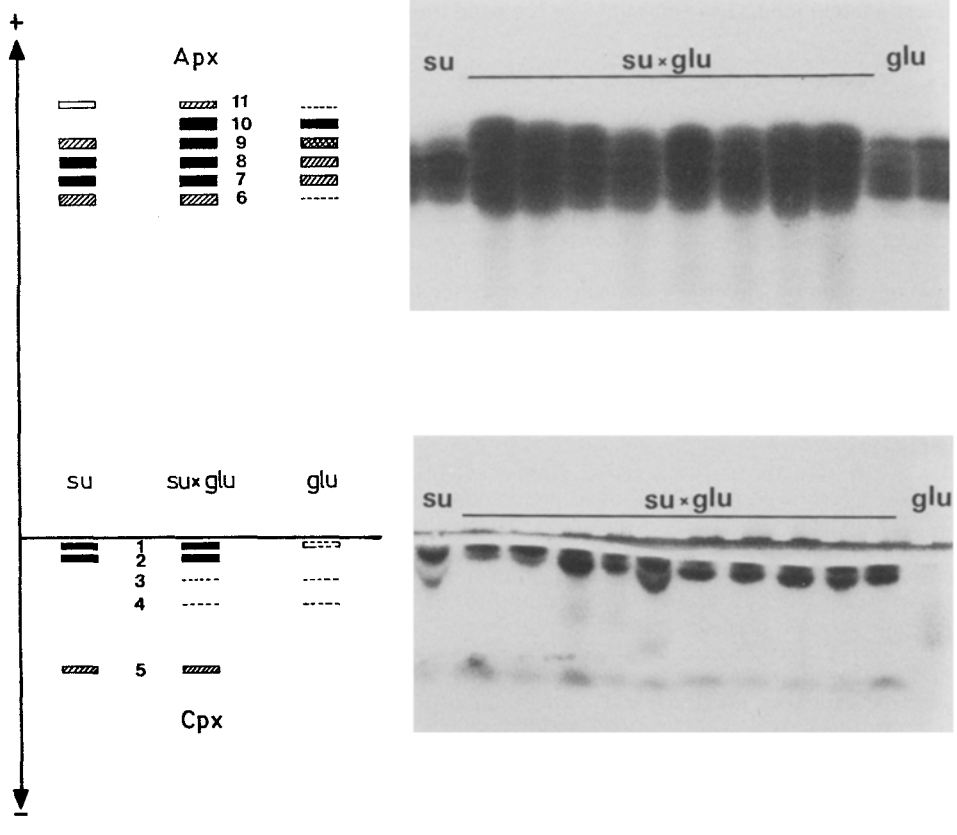

Figure 1. PX patterns for *Nicotiana suaveolens* (su), *N. glutinosa* (glu) and their interspecific hybrid (su × glu) in the leaves corresponding to the level 1 (basal rosette).