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Some aspects of karyotype evolution in *Liliiflorae:* heterochromatin variation and ecology in *Allium pulchellum*

Abstract

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In several genera of the *Liliaceae,* many species are able to generate and tolerate large numbers of heterochromatic segments composed of highly repetitive DNA of AT- and/or GC-nucleotide base composition. These segments are mainly in substitution but also in addition to the euchromatic genome. Their occurrence, number and type is variable between and within species and populations, showing correlation with altitude and/or habitat. A cytological survey of eight altitudinally separated populations of *Allium pulchellum* ($2n = 2x = 16$) has shown that there is an increase in the amount of heterochromatin in higher altitude populations. The increase involves all types of heterochromatic segments present in this species. All the evidence indicates that heterochromatin may have an important adaptive role in *Allium pulchellum,* enabling it to colonize higher ground.

Introduction

In spite of all the progress made possible in cytogenetic studies by the introduction of techniques for the linear differentiation of chromosomes, the reasons for the development of heterochromatin, and indeed of its very existence, are not yet clear. However, evidence from several studies, in various organisms, has shown that heterochromatin can have some functions and that these may be different according to its quantitative and sometimes qualitative properties and its position on the chromosomes.

The present paper reports the results of a study of eight populations of *Allium pulchellum* G. Don. It represents an extension and an elaboration of a cytogenetic survey of this species growing in the region of the Monti Pisani, NE of the city of Pisa, Tuscany (Italy), started by the author in 1974. It includes some already published data (Vosa 1976) relative to three of the same populations (n^{os} 1-3).

Table 1. Constitutive heterochromatin content as a percentage of total karyotype length in eight populations of *Allium pulchellum*.

N° Population	*n° of plants*	*altitude (m a.s.l.)*	*heterochromatin %*
1. Selletta di Agnano Pisano	10	450	20.0
2. Verruca di Calci, Pisa	12	540	21.5
3. Massarosa, Lucca	8	150	17.5
4. "Al Polacco", S. Giuliano T., Pisa	14	200	15.0
5. Cave di S. Giuliano T., Pisa	8	15	9.0
6. Bagnetto di Agnano Pisano, Pisa	18	15	11.0
7. Filettole, Pisa	10	25	9.5
8. Caprona, Pisa	15	30	10.0

Materials and methods

The materials consisted of bulbs collected in the wild at the stage of withering of the flowering stem leaves, when the bulbs are fully grown, in order to ensure vigour in cultivation. All resulting plants were grown in the same potting compost (John Innes n° 1) in plastic pots in a cold greenhouse on the roof of the Department of Plant Sciences of the University of Oxford, England.

For the cytological preparations, actively growing root tips were collected in February and pre-treated in an aqueous solution of 0.05 % colchicine, at the same temperature as the potting compost, for four hours. Fixation was generally in 1 : 3 acetic alcohol overnight. Quinacrine fluorescence staining and C-banding were performed according to the schedule suggested by Vosa (1973).

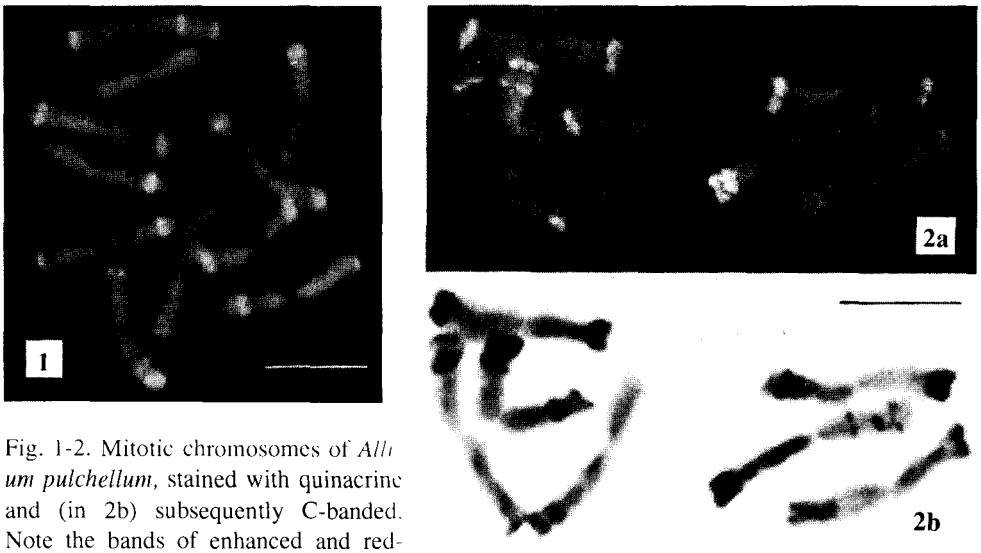

Fig. 1-2. Mitotic chromosomes of *Allium pulchellum*, stained with quinacrine and (in 2b) subsequently C-banded. Note the bands of enhanced and reduced fluorescence (1, 2a) and their correspondence with the C-bands (2b). – Scale bar: 10 μm.

Table 2. Mean number of enhanced (E) and reduced (R) Q-fluorescence bands, C-bands and percentage of heterochromatin in high- and low-altitude populations of *Allium pulchellum*.

Populations	*enhanced bands*	*reduced bands*	*C-bands*	*heterochromatin %*
high-altitude	30	24	50	18.5
low-altitude	22	15	35	9.9

Observations of the quinacrine treated slides were made with a Zeiss Ultraphot II microscope, using a BG 12 exciter filter and a 50 barrier filter. All measurements were taken on enlarged photographic prints from negative micrographs taken on Kodak Tri-X Pan 400 ASA black-and-white film for fluorescence, and Kodak Microfile type film for the C-banded preparations.

Results

The number of plants, localities of collection, altitudes, and percentages of heterochromatin are shown in Table 1. A total of 99 plants in eight population samples of 8 to 18 plants per populations have been studied.

When stained with quinacrine, the heterochromatic segments of *Allium pulchellum* are readily distinguished in AT- (enhanced fluorescence) and GC- (reduced fluorescence) nucleotide base composition (Weisblum 1973). C-banding does not discriminate between enhanced and reduced fluorescence bands but stains both in the same way (Fig. 1-2).

As the data in Tables 1-2 show, there are notable differences in heterochromatin content between the high- and the low-altitude populations. In particular, the samples collected at low altitude show consistently a lower heterochromatin content in comparison with those collected at higher altitude.

This difference is remarkable because of the relatively short distances between the populations which, it could be argued, do not preclude cross-pollination. Thus, in its range on the Monti Pisani, *A. pulchellum* may be behaving as a single interbreeding population. Therefore, the reasons for the differences in heterochromatin content must be found in a probable adaptive role, with the selective effect of altitude influencing seedling survival and successful establishment.

Discussion

In the classical definition of Heitz (1929), heterochromatin consists of chromosome material which maintains its anaphase condensation throughout interphase. it has been further defined as dispensable, non-transcribing, generally repetitive DNA preserved in the genome by natural selection and location and located in the chromosomes as discrete, inheritable segments.

Heterochromatin has no recognizable phenotypic effects and great quantitative and qualitative variability. This implies that it cannot have any indispensable function and that its role may be to influence processes which can be performed adequately in its

absence. In some cases its role seems to be that of a compensating factor influencing cell cycle duration and the relative speed of growth (Nagl 1974, Vosa & Stergianou 1990).

Another reason for the presence of heterochromatin can be found in its effects on genetic recombination, which are known to occur in plants (Rhoades 1978) and in animals (Miklos & Nankivell 1976).

In several genera of the *Liliiflorae,* many species are known to generate and tolerate large amounts of heterochromatin in segments of various size and location on their chromosomes. These H-segments may be in addition to and/or in substitution of the euchromatic genome (see Vosa 1985 for review). Their occurrence, variable in number and type beaten species and populations, has been found to show correlation with habitat and/or altitude (Vosa 1976, 1985, Vosa & Stergianou 1990).

The analysis of population samples of *Allium pulchellum* collected at various altitudes suggests that heterochromatin may have an important adaptive function, enabling the species to colonize relatively higher ground.

Regarding the qualitative functions of heterochromatin, Wolf (1968) was able to demonstrate that in the fly *Phryne cincta* the two types of heterochromatin present in this insect had two distinct kinds of effect: α-heterochromatin on growth and development and β-heterochromatin on meiotic recombination.

As far as the two types of heterochromatin found in *Allium pulchellum* are concerned, their apparent parallel variability, showing only a bias towards an increase with altitude (Table 2), does not bear out a possible differential role.

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