Polyploidy and the critical size of natural populations: the case of cocksfoot (*Dactylis glomerata* L.), a grass used as a fodder plant

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Abstract

Lumaret, R.: Polyploidy and the critical size of natural populations: the case of cocksfoot (*Dactylis glomerata* L.), a grass used as a fodder plant. — Bocconea 7: 133-139. 1997. — ISSN 1120-4060.

Many cultivated plants are polyploids and possess both polyploid and diploid wild relatives. Using as an example the polyploid complex of *Dactylis glomerata* L. (a grass species used partially as a fodder crop), it is shown that ploidy level should be taken into account in formulating strategies for the conservation of the wild relatives of cultivated plants. In association with other biological characteristics, more particularly the reproductive system, the 'ploidy level will determine the extent of seed stocks, the minimum size of population for *in* situ conservation and the nature and degree of disturbance which can be tolerated by the wild populations.

Introduction

Many cultivated plants belong to polyploid complexes within which they usually occupy the higest 'ploidy levels, following natural or artificial polyploidization. The lower levels of these complexes (particularly the diploids) consist almost exclusively of related wild forms of older origin. When it produces a successful result through evolution, apart from the automatic increase in cell size in relation to the amount of DNA, the polyploidization process most often results in an increase in genetic diversity and its maintenance in a fixed or unfixed state (Stebbins 1985, Thompson & Lumaret 1992, Soltis & Soltis 1993). The amount of interaction between the genes also increases. These genetic modifications have effects on the physiology of the organisms (Tal 1980) and hence on the extent of their ecological niche. There are often changes in the length of the biological cycle (vegetative and reproductive periods), the type of reproduction, stress resistance (generally improved), greater ability to cope with environmental disturbance, better capacity for competition and, finally, often improved capacity for colonizing new habitats

(Lumaret 1988a, Thompson & Lumaret 1992). In fact, the colonizing ability of particular plant organisms and the probability that it will become extinct depend directly on the adjustment capacity of the genetic structures of their populations in relation to the extent of variation of the environment. Because of greater genetic diversity and improved physiological balance, polyploids are often less likely to become extinct than related diploids in disturbed habitats.

This is why it seems necessary, in polyploid complexes, to take into account specifically the 'ploidy level of the organisms in question in strategies for the conservation of the wild relatives of cultivated plants. In association with other biological characteristics, particularly the reproductive system, the 'ploidy level will determine the extent of seed stocks, the minimum size of populations for *in situ* conservation and the nature and degree of disturbance which can be tolerated by the populations.

The case of cocksfoot (*Dactylis glomerata* L.), a natural infraspecific polyploid complex, is presented as an example in the present paper. Only a very small fraction of this complex has been used (at tetraploid level only), and found under cultivation as commercial varieties of this fodder grass.

Geographical distribution and population dynamics of *Dactylis glomerata* subspecies in Europe

The cocksfoot allogamous complex has a primarily Eurasian distribution, including the Mediterranean basin. In Europe, it comprises essentially eight diploid sub-species ($2n = 2x = 14$), several of them involving also a few tetraploid morphologically indistinguishable plants which grow in the same areas (Lumaret 1988b), and, at least, five tetraploid subspecies ($2n = 4x = 28$), most of them widely distributed. Each subspecies has a different morphology and a specific geographical distribution (Stebbins & Zohary 1959, Borrill 1978, Lumaret 1988b). Most of the diploid organisms are of very ancient origin and may date back to the Miocene period (20 to 30 million years ago) (Stebbins & Zohary 1959, Borrill 1978). They each have a limited distribution area and a narrow range of adaptation (see Table 1 for their location). They are present in undergrowth in temperate woodland (e.g. subspecies *aschersoniana* and the Galician type), subalpine meadows (e.g. subspp. *reichenbachii* and *juncinella* and the type growing in the centre of Grand Canary island), and certain particular biotopes such as forest margins or clearings (e.g. subspp. *lusitanica*, *ibizensis*, *smithii* and several related types) or rock cavities in Mediterranean or subtropical regions. These very specialised diploid organisms are also strongly distinguished genetically, as has been checked again recently by using genetic allozyme markers (Lumaret 1988b), showing that favourable allele combinations, specific to particular environmental conditions, probably exist (Lumaret 1984, 1988b). These diploid subspecies can therefore be considered genetically as 'relatively pure types', showing potentially very interesting agricultural characteristics in well-defined environmental conditions (Borrill 1978). Most of them, however, have proved too sensitive to human disturbance to be used directly as cultivated varieties.

The geographical distribution of the diploids has receded considerably, especially in the past few decades, essentially because of disturbance and the destruction of biotopes (by clearing, urbanisation and the introduction of certain farming practices). Some subspecies are obviously on the way of extinction (Lumaret 1988b, Sahuquillo & Lumaret 1995).

Table 1. Climate preferences, area, ploidy level and geographical distribution of *Dactylis glomerata* L. subspecies in Europe.

Climate	Subspecies	Ploidy	Geographical Distribution
Temperate	*aschersoniana*	2x (4x)	North-Eastern Europe
	lusitanica	2x	North central Portugal
	Galician type	2x / 4x	Central Galicia (Spain)
	reichenbachii	2x / 4x	Alps (Italy, France, Switzerland)
	slovenica	4x	Slovenia
	glomerata	4x	Cosmopolitan, includes also cultivated varieties
Mediterranean	*juncinella*	2x (4x)	Summit of Sierra Nevada (Spain)
	hispanica	4x	Cosmopolitan
Subtropical	*ibizensis*	2x (4x)	Ibiza and Formentera (Spain)
	smithii	2x	Tenerife (Spain)
	and related forms	4x	La Gomera, La Palma, Gran Canaria, Lanzarote and Hierro, Canary Islands (Spain)
	Canarian type	2x / 4x	Central Gran Canaria island
	hylodes	4x	Madeira (Portugal)
	marina	4x	Mostly coasts of Portugal

Of the eight European subspecies (two of them, namely the Galician and the Gran Canarian types, have not been described formally), three have regressed greatly due to clearing (subspecies *aschersoniana* in north-eastern Europe, *lusitanica* in Portugal, and the Galician type in Spain). Three others have shrunk greatly or almost disappeared due to the spread of tourism-linked urbanisation (these are subspecies *ibizensis* endemic to the Spanish islands of Ibiza and Formentera, *smithii* which is endemic to the Spanish island of Tenerife and, to a lesser degree, the Alpine subspecies *reichenbachii* known in the diploid state in Austria and more particularly in Italy). In addition, the diploid type endemic to the central high elevation area of the island of Gran Canaria (Sahuquillo & Lumaret 1995) is now reduced to a single population including less than hundred individuals, due to the combination of drier climate conditions and overgrazing. Only a single European diploid subspecies seems to be less threatened, surviving, at least in the higher ranges of its distribution which have not been disturbed too much: this is subsp. *juncinella* which is present only at an altitude of between 2 000 and 3 000 metres in the Sierra Nevada located in the south of Spain.

In cocksfoot, most of the natural so called 'cosmopolitan' tetraploids which developed chiefly during the Quaternary period and particularly during the last 10 000 years, have high genetic diversity, both in individuals and in populations (Lumaret 1988b). These tetraploids are spread over a wide range of habitats (though less specialized and less extreme than for the diploids); they display continuum of forms intermediate between climate ecotypes (Knight 1973, Glaszmann & al. 1982, Lumaret & Hanotte 1987) and, compared with the diploids, seem to be clearly favoured, in placed affected byby human activities, especially more open habitats. Apart from natural grassland, the tetraploid cocksfoot that is one of the cosmopolitan types is often observed along the verges of cultivated fields and tracks. This 'cosmopolitan' type of cocksfoot generally possesses the biological characteristics and genetic diversity of several diploid subspecies and shows great phenotypic plasticity (Borrill 1978, Lumaret 1988b). The commercial varieties of cocksfoot are derived mainly from a single cosmopolitan humid temperate climate tetraploid subspecies (subsp. *glomerata*) which has a fairly restricted genetic base as compared with the species as a whole.

In addition to the cosmopolitan tetraploids, tetraploids morphologically indistinguishable from a particular sympatric diploid type have been observed for virtually all European subspecies (except subsp. *lusitanica*) (Lumaret 1988, Sahuquillo & Lumaret 1995). We now know that the production of 2*n*-gametes (both male and female) in diploids is responsible for autotetraploidisation of cocksfoot in nature (Borrill 1978, Lumaret 1988b, Lumaret 1991, Maceira & al. 1992, De Haan & al. 1992). Whereas the cosmopolitan tetraploids are in full expansion because of their good tolerance of disturbance and their growing use as a fodder crop, the same is not true of the tetraploids that are directly related to particular diploids which, although they are already differentiated from the latter and possess higher genetic diversity, owe their integrity merely to geographical or temporal isolation (different flowering times) preventing hybridisation with other neighbouring tetraploid types (Ardouin & al. 1987, Lumaret 1988b, Jay & al. 1989, Lumaret & Barrientos 1990, Sahuquillo & Lumaret 1995). It can therefore be concluded that the most important factor in the disappearance of this type of tetraploid forms is the occurrence of contact with other types of cocksfoot of the same 'ploidy level and, particularly, with the cultivated varieties introduced with increasing frequency into areas in certain regions of Europe which hitherto used local hay. This type of situation and its short- and medium-term consequences have been described in the Swiss Alps and especially in Galicia (Lumaret & Hanotte 1987, Lumaret 1990). In the case of the island of Gran Canaria, where isolation and especially the climate do not allow cocksfoot introduced from continental Europe or North Africa to survive, the virtual disappearance of the endemic tetraploid type from the centre of the island (only three stations only remain) is due to overgrazing linked with rural overpopulation of the island.

Decreases in farming activities during the two last decade has encouraged repopulation by this cocksfoot type using appropriate measures (protection of areas and seed multiplication). According to our recent survey in that region (late 1994), a substantial increase of population size in the tetraploid Gran Canarian cocksfoot could be obseved, at least in one local protected area.

Reproductive constraints in cocksfoot

Observations on populations *in situ* over several consecutive years has made it possible to identify several factors and processes leading to the regression of the diploid subspecies. These constitute entities for which vegetative cycle and period of reproduction, whether annual or biannual, are closely linked to habitat conditions. For example, subspecies *aschersoniana, reichenbachii, juncinella* and the Galician type are late- flowering (August), which means that reproduction will be successful only if air humidity at that time is adequate to maintain pollen fertility. Humidity is provided either by vegetation cover or by high elevation (Lumaret & al. 1987, Lumaret 1988b, Lumaret & Barrientos 1990). When clearing takes place, the plants are suddenly subjected to open (dry) conditions and can no longer reproduce in the summer. Experiments in controlled conditions have also shown that the diploids have poor ability to withstand competition as compared with the polyploids of the same subspecies (Maceira & al. 1993), which forces them to remain in restricting habitats (such as undergrowth where the amount of light is limited) and therefore selective versus other plant species. Furthermore, clearing and urbanisation result in fragmentation and reduction of population size. For this allogamous species which has a self-incompatible genetic system (Lundquist 1965), and is very sensitive to inbreeding depression (Hanson & Carnahan 1956), chiefly at the diploid level, effects of inbreeding and genetic drift are felt quickly and are probably an important factor contributing to the disappearance of diploid populations. According to our observations, in small sized diploid populations of cocksfoot, seeds have usually a very low germination rate and a high proportion of seedlings show chlorophyll-deficiency that is a recessive character.

Management strategies and monitoring in the *Dactylis glomerata* complex

From the given information above, it seems that an effective conservation strategy for wild cocksfoot should involve:

(1) Because there is little genetic variation between diploid populations of the same subspecies, a very small number of protected sites will be is enough, provided that a sufficiently large population is maintained (several tens of thousands individuals.. In addition, stocks of seed could be preserved at a low temperature for periods as long as possible (a factor which is poorly controlled at present), possibly associated with multiplication in protected biotopes. In the case of subspecies restricted to a very low number of populations (sometimes a single one) each including only very few individuals, polycrosses involving as many plants from as many sites as possible are necessary to restore genetic diversity and hence plant fertility and vigour.

(2) For tetraploid populations that are related to a single well-defined diploid or constitute specific ecotypes, the maintenance of biotopes protected against contacts with other tetraploids is essential. On the other hand, the size of populations under in *situ* conservation may be smaller than that needed for the diploids because tetraploids retain genetic diversity due to the high level of genetic inertia linked to tetrasomy.

(3) In addition to *in situ* conservation of the genetic diversity present in cocksfoot, dynamic *ex situ* management of that diversity can be suggested. It entails developing, for agronomic use, new tetraploid types in regions where current commercial varieties are maladapted or else where local tetraploid ecotypes exist. In the case of specific tetraploids which, in the main, have lost their specific nature recently because of the introduction of cultivated material, it is possible to produce new tetraploid material having particular characteristics by direct sexual polyploidisation from diploids, using the $2n$-gametes method. A successful operation of this type has already been carried out experimentally from the Portuguese diploid subspecies *lusitanica* (Bretagnolle & Lumaret 1995) and could be extented to other diploid subspecies.

The conservation strategy for cocksfoot could also be applied to other polyploid complexes.

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