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Genetic diversity, reproductive biology and conservation strategies of endangered species

Abstract

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The authors examined the relationship between the amount of genetic diversity and some biological and populational characteristics (ploidy level, ratio of allogamy vs. autogamy, population size, existence and distance of other populations of the same species). Some case studies served to demonstrate that genetic diversity cannot be reliably predicted *a-priori*, because it depends on the interaction of several factors. The importance of historical factors in determining infraspecific diversity is stressed.

The authors conclude that reliable (i.e. directly measured) estimates of genetic diversity, both within populations and within individuals, are necessary in order to determine if a plant species is in need of conservation, to define conservation strategies, and to select germplasm sources for *in situ* or *ex situ* conservation.

Introduction

The present contribution is focused on the relations between direct measures of genetic diversity and its prediction based on reproductive biology. Particular attention is given to the consequences on conservation of rare species.

In any plant species there is an obvious relation between population structure, reproductive biology, and genetic diversity. The issue becomes crucial in the case of rare species, where genetic diversity is connected to the state of endangerment, and to the definition of effective conservation strategies.

It is generally assumed that the more restricted and/or fragmented is the geographic distribution of a species, the smaller is its genetic diversity. Also, it has been often stated that the richer is the genetic diversity, the more are the chances of survival (Frankel & al. 1995). Both these tenets, though, have been repeatedly challenged.

Indeed, real situations are complex, and can hardly be reduced to straightforward schemes.

"Genetic diversity" is the molecular basis of biodiversity. It may be defined as the rich-

ness in different alleles in an individual plant, in a population, or in a species. Therefore, genetic diversity has a hierarchical structure (Fig. 1)

If we assume the population as the basic biological unit, both from the point of view of evolution and of conservation, than the amount of genetic diversity stored in the population is of the highest importance for the management of biodiversity. On the other side, assessing how diversity is partitioned among individuals is an essential premise in order to select the best sources of germplasm for conservation.

The following paramenters are commonly used to describe and quantify genetic diversity:

P: % of polymorphic loci , where a locus is considered polymorphic if the most frequent allele does not exceed 95% ($P_{0.95}$) or 99% ($P_{0.99}$).

A: mean number of alleles per locus.

$\mathbf{H_{exp}}$: expected heterozygosity, which is computed for each locus, based on the frequency of its alleles. High values of H_{exp} are indicative of equi-partition of the genetic diversity within the genome.

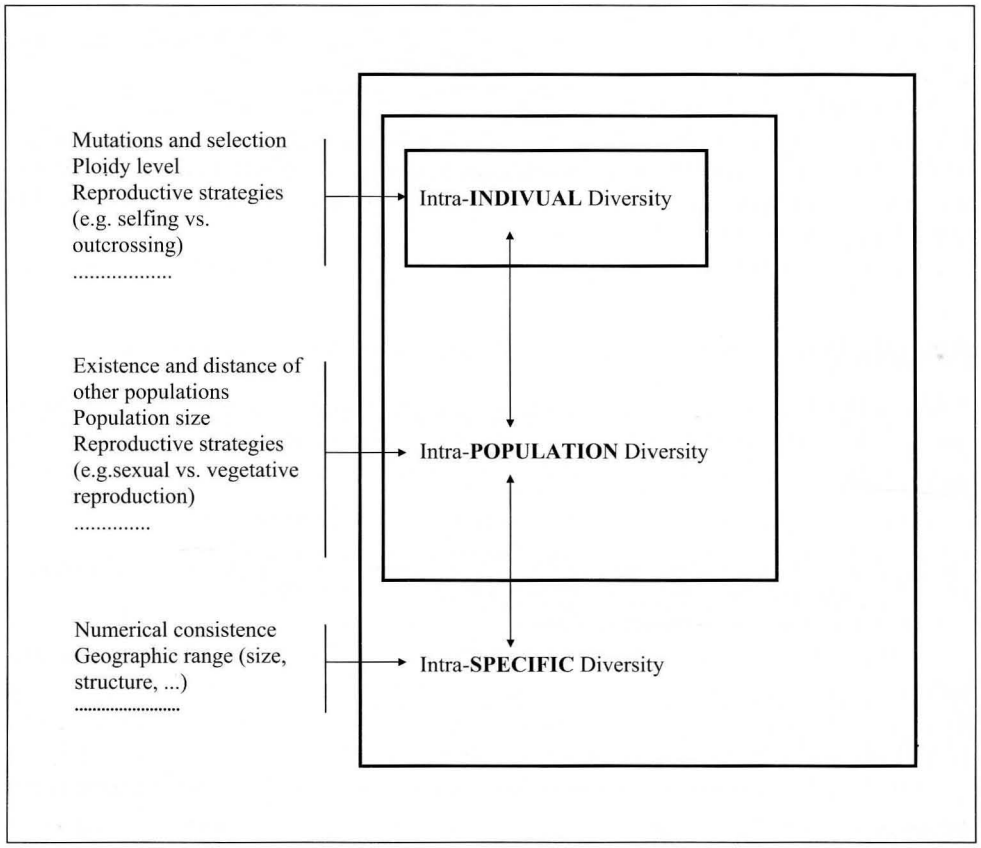

Fig. 1. Hierarchical structure of genetic diversity.

H_{obs}: observed heterozygosity, which is the amount of heterozygous loci actually observed. It may differ from the expected value, mainly in relation to reproductive strategies.

F: fixation index, which is derived from the two paramenters above. In the case of perfect panmixis, under Hardy-Weinberg equilibrium, F equals zero; it varies above zero if the rate of homozygotes is higher than expected, below zero if it is lower than expected.

The choice of molecular markers

Measuring genetic diversity is a critical issue. Most data on genetic diversity published during the past decades were derived from isozyme patterns, while nowadays DNA polymorphism is being increasingly used to the same aim.

The two molecular markers are not equivalent, as the degree of polymorphism and of heterozygosity estimated through Random Amplified Polymorphic DNA Fragments often appear higher than the same parameters measured by enzyme diversity.

An example is presented in Table 1, where estimates of genetic diversity of the same population obtained using two different methods are compared. The values of P, H, and F estimated through allozyme analysis were significantly lower than the same parameters estimated using RAPDs.

Similar results were reported by Isabel & al. (1995); these authors suggested that the difference can be due to the fact that allozyme variability is subject to natural selection, while random amplified polymorphic DNA loci are likely to fall within noncoding DNA, which is more subject to random variation. Therefore, the two different estimates have different biological meaning.

All data presented in the following part of this paper were derived from allozyme analyses.

How does diversity correlate with the biological characteristics of a species?

The correlation between genetic diversity and reproductive biology was checked on four species, three Legumes and one Conifer. One population was studied for each species.

1. *Cytisus villosus* Pourr., a species widely distributed from Morocco to the central

Table 1. Comparative data of genetic diversity of Abies nebrodensis. Allozyme data from Vicario & al. (1995). RAPDs data from Conte & Cristofolini, unpubl.

	$P_{0,05}$	H_{exp}	H_{obs}	F
12 allozymes(25 alleles)	0.58	0.14	0.17	- 0.21
6 primers (374 DNA fragments)	0.91	0.37	0.49	- 0.30

Mediterranean basin. Genetic diversity was measured in an isolated population on the Isle Salina, Eolian Archipelago (Troia & al. 1998).

2. *Cytisus emeriflorus* Reichenb., an endemic of Italian Pre-Alps, ranging from Tessin to Friuli. The data were obtained from a population at Passo della Presolana (Conte & Cristofolini 2000).

3. *Cytisus aeolicus* Guss., an endemic of the isles Stromboli, Vulcano and Alicudi, Eolian Archipelago. Data were obtained from the only relatively numerous natural population, on the isle Stromboli (Conte & al. 1998).

4. *Abies nebrodensis* Lojac., an endemic of the Madonie Mountain range, not far from Palermo. The data presented were obtained from the only natural population of this species (Conte & Cristofolini unpublished data).

Ploidy level.- The upper limit of alleles per locus in a diploid plant is A = 2, while in a tetraploid the theoretic upper limit is A = 4. Therefore, polyploids are expected to present an average number of alleles per locus higher than diploids, and to some extent it is so.

However, only rarely we found more than two alleles on the same locus, even in tetraploids. Also, it happens that diploids have higher indices of allelic diversity than tetraploids (Table 2).

Reproductive strategy.- As a general rule, it has been shown that selfing, while not affecting the genic richness of the population, decreases the individual variability, determining a higher proportion of homozygosis.

Of the three Leguminous plants, *C.villosus* and *C.emeriflorus* are certainly allogamous, while some doubt exist for *C.aeolicus*. The conifer *Abies nebrodensis*, of course does not have any means of preventing selfing. Therefore, it has been speculated (e.g. by Ducci & al. 1999) that selfing should be prevailing, due to the low number of flowering specimens and to the distance from one another.

This assertion proved not true (Table 3).

In the three species of *Cytisus* the observed heterozygosity only slightly deviates from the expected value; the Fixation index close to zero indicates a situation of full panmixis,

Table 2. Relation between ploidy level and alleles per locus.

	Cytisus villosus **Salina**	***Cytisus emeriflorus*** **Presolana**	***Cytisus aeolicus*** **Stromboli**	***Abies nebrodensis*** **Madonie**
Ploidy level	2n = 4x	2n = 4x	2n = 4x	2n= 2x
Alleles per locus	1,45	2,00	1,31	1,58

Table 3. Relationship between reproductive strategy and heterozygosity.

	Cytisus villosus **Salina**	*Cytisus emeriflorus* **Presolana**	*Cytisus aeolicus* **Stromboli**	*Abies nebrodensis* **Madonie**
Pollination system	Entomophylous (mainly bees)	Entomophylous (mainly bees)	Entomophylous (mainly bees)	Anemophylous
Self-compatibility	Self-incompatible	Self-incompatible	Self-incompatible?	Selfing and outcrossing possible
Expected heterozygosity	0,19	0,44	0,27	0,35
Observed heterozygosity	0,19	0,46	0,29	0,46
Fixation index	0,05	- 0,04	- 0,09	- 0,21

under Hardy-Weinberg equilibrium. By contrast, Fixation index in *Abies nebrodensis* is negative, with an absolute value remarkably high, indicating an excess of heterozygotes, i.e. an excess of outcrossing compared to selfing.

Although this result may appear difficult to be explained, a similar situation has been observed in *Picea abies* (Isabel & al. 1995). In that case, it has been argued that the excess of heterozygotes observed in mature populations is not likely to be explained by over-dominance hypothesis. Rather, selection against deleterious alleles found in homozygotes arising from consanguineous matings is a more likely hypothesis. This hypothesis is supported by the excess of homozygotes found in seed and juvenile populations of the same species (Knawles 1985; cited by Isabel & al. 1995).

Apart from any speculation about the mechanisms that generate the low rate of homozygotes, we can state that pollination biology alone is not a good predictor of the ratio homozygotes vs. heterozygotes in the adult reproductive population.

Table 4. Relationship between population size and genetic diversity.

	Cytisus villosus **Salina**	***Cytisus emeriflorus*** **Presolana**	***Cytisus aeolicus*** **Stromboli**	***Abies nebrodensis*** **Madonie**
Estimate population size	x .10 ^{3}	x . 10 ^{2}	x . 10 ^{2}	x . 10 ^{1}
Alleles per locus	1,45	2,00	1,31	1,58
% polymorphic loci	27,0	63,6	31,3	58,3
Expected heterozygosity	0,19	0,44	0,27	0,35

Population size.- The genetic variability (A, H_{exp}, $P_{0,05}$) of panmictic species is expected to be positively correlated to population size.

In fact, it is not necessarily so (Table 4). The three leguminous species are biologically similar to each other, share the same reproductive system, the same ploidy level, and are taxonomically related. Nevertheless, there is no correlation between number of individuals in their populations and the main parameters of genetic diversity.

Distance to the nearest population.- A comparison among the three Leguminous species, which are biologically similar, suggests that the existence and the distance of other populations may act as a source of allelic diversity, and allow an increase of overall diversity (Table 5). All parameters of genetic diversity (A, H_{exp}, $P_{0,05}$) are positively correlated to the distance of the nearest population.

Discussion

Several factors are related to biodiversity. No single factor can fully account for it, as biodiversity results from their mutual interaction.

Table 5. Relationship between distance to the nearest population and genetic diversity.

	Cytisus emeriflorus **Presolana**	***Cytisus villosus*** **Salina** (in parenthesis averaged values of 5 populations of the main dis-tribution range)	***Cytisus aeolicus*** **Stromboli**
Distance (km) to the nearest population	ca. 20	ca. 50	==
% polymorphic loci	63,6	27,0 (34,2)	31,3
Alleles per locus	2,0	1,45 (1,57)	1,31
Expected heterozygosity	0,44	0,19 (0,24)	0,27

Moreover, historical factors cannot be overlooked, although they cannot be measured or quantified.

The case of *Cytisus aeolicus* and *Abies nebrodensis* is emblematic. Both species are endemites with an extremely narrow distribution. *Abies nebrodensis* is reduced to a number of living specimens lower than *Cytisus aeolicus* of one order of magnitude: some tens rather than some hundreds. Nevertheless, the former has a genetic diversity far higher than the latter. The key to this question can be found in history.

Cytisus aeolicus certainly suffered repeated semi-extictions during Pleistocene, due to volcanic events that may have reduced its populations to few specimens. The living population is the outcome of one (or more) bottleneck, that drastically reduced its genetic diversity.

Abies nebrodensis, by contrast, underwent a drastic numerical reduction only in historical times, and perhaps underwent some introgression with *Abies alba* (Raimondo & al.

1990). The individual diversity indicates that the few living plants of this species contain an amount of genetic diversity that was relatively well preserved until recent times. As a result, in spite of the reduced population size, the extant 29 trees of *Abies nebrodensis* may have better chances of recover than the ca. 300 *Cytisus aeolicus* plants.

Comparing a small population of the endemite *Cytisus emeriflorus* with a larger population of *Cytisus villosus* is also illuminating. In spite of population size, the former contains more biodiversity than the latter.

The fragmentation of *Cytisus emeriflorus* range seems to be holocenic (Conte & Cristofolini 2000), possibly related to human impact, so that the small extant populations still are, genetically, a part of a broader metapopulation. On the contrary, the insular population of *Cytisus villosus* certainly did not have any contact with the mainland populations after its foundation, and maintains the reduced variability inherited through the initial bottle-neck.

As a general conclusion, we may state that accurate estimates of genetic diversity are necessary to define the condition of danger of rare species, and to select the most appropriate sources of germplasm. In spite of some criticism (e.g. by Frankel & al. 1995) we agree with Falk (1990) that monitoring of genetic variation is a preliminary task for the conservation biologist.

The genetic diversity cannot be predicted solely on the basis of the biological or phytogeographical characteristics of a species. There is, of course, a general correlation between e.g. population size and genetic diversity, or between selfing and homozygosis; however, there is such a variability from species to species, so many and different are the interacting factors, so important are the past events, that reliable estimates can be only obtained through direct experimental measures.

Effective, rapid, and rather simple techniques (isozyme analysis, DNA polymorphism etc.) are available to obtain direct measures of genomic diversity at any taxonomic level, so that a task, that only a few years ago seemed hard to be accomplished, has become feasible. The results are certainly worth the effort required.

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