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Assessment of the minimum sample size required to characterize site-scale airborne pollen

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Many palynological investigations require the comparison of large collections of samples and here the optimization of the effort is crucial. A method to determine the pollen sum according to the intrinsic characteristics of the site pollen composition is proposed. Different variables such as pollen spectra, typological lists, richness, diversity, intra- and inter-sites affinities, are alternatively analyzed in order to determine the minimum sample size and the results are compared. An example of this methodology is developed for the airborne pollen from an agroecosystem in the Pampean grasslands. Increasing pollen counts from 150 to above 2700 does not yield different results among the dominant and subdominant types, which account for 70%–80% of the pollen sum. Both diversity estimates and similarity among sites are not significantly affected when quantitative coefficients are employed. As pollen counts increase, there is an increment in the number of types, but the types added with counts over 150 are always rare, their overall relative frequency never exceeding 6%. The minimum sample size obtained as shown here provides the necessary information to reconstruct the major pollen fraction of the site and it provides reliable estimates of the typologic diversity and the affinities among sites.

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Many palynological investigations require the analysis of a large number of samples; thus making the optimization of effort crucial. During counting, the gain of information is a function of sample size. In consequence, the selection of a minimum sample size depends both on the objectives and on the spatial scale of the research (Janssen 1980, Hicks 1985). It is critical to differentiate between local (site) and the regional spectra, two relevant scales in palynological studies (Faegri & Iversen 1975, D'Antoni 1979). The characterization of local pollen is an essential step for further regional studies and several papers have been centered on site-scale spectra (e.g. Andersen 1970, Birks 1973, O'Sullivan 1973, Hicks 1985).

The achievement of a site pollen spectrum implies the production of either a taxonomic or a typological list, and the estimation of the frequency distribution of the taxa deposited in a determined site during a known period of time. The reliability of the site characterization is strongly related to the sample size (or pollen sum). A review of previous work shows that there are different criteria in selecting the size of the pollen sum, ranging from convenience (e.g., Faegri & Iversen 1975, Moore & Webb 1978, D'Antoni 1979) to statistical considerations (e.g., Mosimann 1965, Maher 1981, Hill 1996). Many papers have focused on palaeoecological and/or AP (arboreal pollen) analysis (e.g., Bowman 1931, Hafsten 1956, Moore & Webb 1978, Birks & Birks 1980) and, according to their results, the minimum sample size can be established between 150–1500 grains. The generalization of these results to all kinds of pollen analyses would seem to be a strong temptation but, as Faegri & Iversen (1992) pointed out, "there is nothing like one 'correct'

pollen sum; different questions presume different pollen sums even within the same region". In other words, there is no universal standard size for palynological work (Moore & Webb 1978, Birks & Birks 1980, Maher 1981, Hill 1996) and sampling effort depends on the actual research questions and on the site characteristics.

The purpose of this paper is to propose a method to determine the sample size according to the intrinsic characteristics of the composition of the spectrum. According to modern trends (Birks & Birks 1980, D'Antoni & Madanes 1986, Rull 1987), synthetic expressions such as diversity and richness are used in the present paper to evaluate the information gain. Different variables such as pollen spectra, typological lists, richness, diversity, and intra- and inter-site affinities, are selected to determine the minimum sample size and the results are compared in order to minimize the sample size where the optimum relationship of information gain to effort is obtained.

The aerial pollen from an agroecosystem in the Pampean grasslands was chosen for the assessment of the minimum pollen sum, since it presents some convenient properties in time and space. The limits of agroecosystems are artificial and usually more abrupt than natural ones. In addition, while in stratified deposits, a single pollen spectrum represents several cycles (seasonal, annual, etc.), in anthropogenic systems, both seeds and crop develop simultaneously during a very short time. Thus, the genesis of the variability observed can be confined in space and time, and so, only discrete units of the cycle can be analyzed (D'Antoni & Madanes 1986).

MATERIALS AND METHODS

The sampling was carried out in the Experimental Area of the Balcarce Experimental Station, INTA (Instituto Nacional de

Tecnología Agropecuaria) to the south of Buenos Aires, Argentina (37°51'S and 58°15'W). The station is located within the Southern Pampean district of the Pampean region. The natural vegetation of the region is predominantly grasslands, with different species of *Stipa* and *Piptochaetium* (Cabrera & Zardini 1978), but the natural environment has been replaced almost entirely by farms and cattle-ranches since the XVIIIth century. At present, the landscape is dominated by anthropic patches with crops and pastures.

The Experimental Area of the Station (75 m × 100 m) was divided into fields – some fallow, some with cultivars of wheat, maize, and sunflower. The Experimental Area is close to a park (340 Ha) with some groups of ornamental trees (mostly *Eucalyptus* spp.). At a distance of 200 m from the Experimental Area there is a 50 Ha area with relict vegetation dominated by *Paspalum quadrifarium*, with patches of *Blechnum* sp; this vegetation is associated with the presence of a hill.

Four Tauber-type traps were placed 0.75 m above the ground at the corners of the experimental area in December, before the crop-flowering time. They were checked weekly. A vegetation survey in the experimental and neighbouring areas was carried out simultaneously with the pollen sampling, which lasted until the end of the post-flowering period (March).

Each trap content was concentrated by centrifugation and digested successively with 10% KOH, 10% HCl, and cold 70% HF for 24 hours to remove silica. Fluorosilicates formed in this step were removed with hot HCl washing. Residues were placed in 0.5 ml glycerin and stored in test tubes. After thorough homogenization with a vortex, the material was added to glycerin and basic fuchsin for pollen analysis. Generic or, when possible, specific identification of pollen grains was performed; otherwise, pollen types were recognized (Table I). For each site, 19–26 replicate counts of 150 grains were obtained. For the NW site, the count was continued up to 31,100 grains.

For data analysis, two similarity coefficients were used: a binary (presence-absence) coefficient (Sørensen 1948) and a quantitative one, the complement of Euclidean distance (1 – Δ).

Sørensen's coefficient between samples *j* and *k* (S_{jk}) is:

$$S_{jk} = \frac{2a}{2a + b + c}$$

where *a* = number of types in samples *j* and *k*;

b = number of types in sample *j* but not in sample *k*;

c = number of types in sample *k* but not in sample *j*.

The complement of Euclidean distance between samples *j* and *k* (Δ_{jk}) is:

$$1 - \Delta_{jk} = 1 - \left[\frac{\sum_{i=1}^d (x_{ij} - x_{ik})^2}{d} \right]^{0.5}$$

where x_{ij} = number of grains of type *i* in sample *j*;

x_{ik} = number of grains of type *i* in sample *k*;

d = number of types.

The optimum relationship between sampling effort and information gain was estimated by means of a saturation criterion with two different variables; this technique is an extension of the minimum sample area method (Mueller-Dombois & Ellenberg 1974). The first variable is the number of types. The minimum sample size is the point at which the initially steeply increasing curve of number of types versus sample size becomes almost horizontal. The second variable was Rull's (1987), who proposed to use the Shannon-Weaver function *H'* versus the sample size, being

$$H' = - \sum_{i=1}^n (p_i) (\log_2 p_i),$$

where *n* = number of types;

p_i = proportion of total grains belonging to *i* th type.

Rull defined the diversity saturation point (DSP) as the critical point after which the variation of that curve shows a negligible variation, or it shows a more or less horizontal fluctuation.

Pielou's pooled quadrat method was used to estimate the popula-

tion diversity H'_{pop} . It involves repeatedly calculating *H'* on randomly accumulated samples. H'_{pop} is estimated by using the flattened portion of the curve, according to the calculations described in Magurran (1991).

Analysis of variance (ANOVA) techniques (see, for example, Steel & Torrie 1985) were carried out to test the following hypotheses:

H_{01} = There is no significant differences in the means of the diversity values of each sample size.

H_{02} = There is no significant differences in the means of the diversity values of each site.

H_{03} = There is no significant interaction among sample size and site.

The similarity among sites was analyzed by means of cluster analysis techniques. The average-linkage, unweighted-pair group (UPGMA) method in the Q mode was used (Romesburg 1984). Sørensen's coefficient (S_s) and the complement of the Euclidean distance were chosen as similarity coefficients for binary and quantitative analysis, respectively.

RESULTS

Number of pollen types

A total of 49 pollen types was recorded in the experimental area of the Balcarce Experimental Station. *Eucalyptus*, Brassicaceae, Apiaceae, *Taraxacum* type, and *Carduus* type were the most abundant taxa (Table I). Taxonomic and quantitative composition of the pollen spectrum differed with sample size. The typological lists ranged from 10–19 taxa in 150-grain samples to 24–30 in 2800/3900-grain ones. The list continued increasing when extremely high numbers were counted in one site, but not all taxa recorded in the area appeared together in the same site. For example, after a 31,100 grains count, only 36 types were registered in the NW site.

Some types were exclusive to certain sites, as occurred with *Adiantum* type, *Alternanthera*, Algae, Bryophyta, *Cupressus*, Campanulaceae, *Junglans*, *Pinus*, Poaceae 20 μm, and *Spergularia* in the NW site.

Site pollen spectra

The dominant taxon in the SW site for two different sample sizes (150 and 3900 grains) was Apiaceae, while Centaureae was subdominant (Table I). Other types, such as *Xanthium*, *Carduus* type, *Helianthus*, Brassicaceae and Poaceae 35 μm were recorded. These taxa represented altogether 83% of the pollen for *n* = 150 and 75% for *n* = 3,900.

The dominant taxon of the NW site for *n* = 150 was *Eucalyptus* (66%), followed by Brassicaceae, *Carduus* type and *Taraxacum* type (19%). No types with less than 1% were recorded. For *n* = 3,900, *Eucalyptus* was also plainly dominant and together with Brassicaceae they both peaked at up to 81%.

In the SE site, the same dominant taxa resulted for 150 and 3900 grain counts, varying their relative frequency as a whole from 74% to 78%, respectively. A co-dominance of Apiaceae, *Carduus* type, and *Eucalyptus* was observed; Chenopodiaceae and *Ambrosia* appeared as subdominants.

In the site NE, for *n* = 150 the dominant taxon was *Taraxacum* type (33%); *Ambrosia*, *Helianthus*, and Chenopodiaceae (69%) were subdominants. This pattern was repeated for *n* = 2850, even maintaining their total relative frequency (71%).

Table I. Summary results for pollen sum equal to 150 and larger than 2850 grains.

Affinity between samples for each site are estimated with Sørensen's coefficient (S) and $1 - \Delta$ (Δ : Euclidian distance).

Site	SW		NW		SE		NE	
Sample size	150	3900	150	3900	150	3900	150	2850
Number of pollen types	19	30	10	24	16	28	14	28
Dominant types	Apiaceae (36.67%) Centaureae (12%) <i>Xanthium</i> (10.67%) <i>Carduus</i> t. (7.33%) <i>Helianthus</i> (7.33%) Brassicaceae (4.67%) Poaceae 35 μ m (4.67%)	Apiaceae (37.03%) Centaureae (14.28%) <i>Carduus</i> t. (9.28%) <i>Xanthium</i> (7.44%) Brassicaceae (7.05%)	<i>Eucalyptus</i> (66%) Brassicaceae (8.67%) <i>Carduus</i> t. (5.33%) <i>Taraxacum</i> t. (5.33%)	<i>Eucalyptus</i> (69.31%) Brassicaceae (11.90%)	Apiaceae (20%) <i>Carduus</i> t. (18%) <i>Eucalyptus</i> (17.33%) Chenopodiaceae (10%) Ambrosia (8.67%)	Apiaceae (28.41%) <i>Eucalyptus</i> (20.87%) <i>Carduus</i> t. (13.05%) Ambrosia (6.36%) Chenopodiaceae (5%) Brassicaceae (4.51%)	<i>Taraxacum</i> t. (33.33%) Ambrosia (13.33%) <i>Helianthus</i> (12.67%) Chenopodiaceae (10%)	<i>Taraxacum</i> t. (32.14%) <i>Helianthus</i> (16.35%) Ambrosia (12.39%) Chenopodiaceae (10.98%)
Number of pollen types with relative frequency below 1%	7	19	0	16	3	13	3	17
Overall relative frequency	(4.69%)	(5.64%)	(0%)	(2.36%)	(2.01%)	(1.57%)	(2.01%)	(3.17%)
Types added after increasing the pollen sum		<i>Anthemis</i> t. <i>Blechnum</i> t. Caprifoliaceae Cyperaceae <i>Polygonum</i> <i>Polystichum</i> <i>Rumex</i> t. <i>Senecio</i> t. <i>Triticum</i> <i>Viola</i> t. <i>Zea</i>		Centaureae Cyperaceae <i>Helianthus</i> <i>Lotus</i> t. Oxalidaceae <i>Plantago</i> t. Poaceae 50 μ m <i>Polygonum</i> Rubiaceae <i>Senecio</i> t. Solanaceae <i>Tripholium</i> t. <i>Triticum</i> <i>Zea</i>		<i>Baccharis</i> t. <i>Blechnum</i> t. Cariophyllaceae Fabaceae <i>Lotus</i> t. Oxalidaceae Papilionoideae <i>Plantago</i> t. <i>Senecio</i> t. Solanaceae <i>Urtica</i> t. <i>Viola</i> t.		<i>Anthemis</i> t. Cyncreae Cyperaceae <i>Echium</i> t. Fabaceae <i>Lotus</i> t. <i>Plantago</i> <i>Psila</i> t. Ranunculaceae Rosaceae <i>Senecio</i> t. <i>Vicea</i> t.
S		0.77		0.57		0.72		0.70
$1 - \Delta$		0.88		0.91		0.84		0.91

Table IIA. Diversity (*H'*) estimates with samples of different sizes.

Sample size	Replicate	Site SW	Site NW	Site SE	Site NE
150	1	3.17	1.89	3.17	3.04
	2	2.84	1.77	2.86	3.19
300	1	3.12	1.67	3.09	3.03
	2	3.21	1.71	3.11	3.05
600	1	3.00	1.85	3.08	3.00
	2	3.12	1.71	3.09	3.01

In all cases, increasing the pollen sum from 150 to 2800/3900 resulted in the addition of a comparatively large number of new taxa (57–140% of the previous number of types; Table I) but their individual relative frequencies never reached 1%.

Affinity between the two pollen spectra (*n*=150 and *n*=2800/3900) of each site was estimated using a binary coefficient and a quantitative one (see Materials and Methods). With the binary coefficient, pollen spectra showed approximately 70% affinity with the exception of the NW site, where no rare types (less than 1%) were present with *n*=150 (Table I). The estimated affinity calculated with the quantitative coefficient showed values that ranged from 84% to 91% for all the sites.

Number of pollen types vs. sample-size curve

The number of pollen types versus sample-size curve is entirely analogous to the curve of the number of species versus sampling-area used to establish the minimum area relationship (Salgado-Laboriau & Schubert 1976, Capraris et al. 1976, Braun-Blanquet 1979, Matteucci & Colma 1982, Rull 1987, Kent & Coker 1992).

Pollen richness estimates show a great dependence upon sample size (Magurran 1991). The resulting curves theoretically tend to an asymptote, whose value is however difficult to determine with field data. To estimate the number of types in each site, a saturation criterion is used. It consists of following the counting until no new taxa appear after a certain number of consecutive counts (Salgado-Laboriau & Schubert 1976).

From Figure 1 it is obvious that, except in SW site (where the curve is asymptotic beyond 2100 grains), the curves do

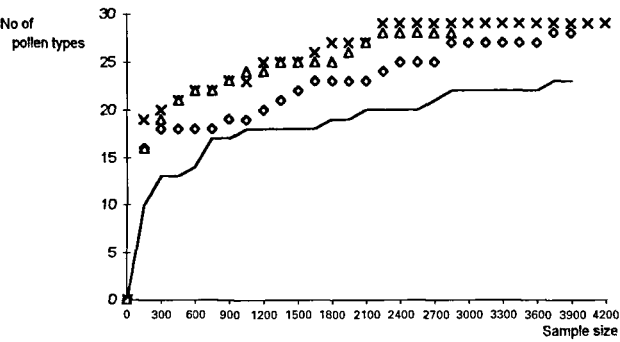


Fig. 1. Richness versus sample size. X: site SW; —: site NW; Δ: site SE; ◇: site NE.

not reach an asymptote; the number of pollen types continued increasing together with an increasingly large pollen sum (3900 grains).

Typological diversity

Diversity measures take into account two factors: richness (number of categories) and evenness (how abundant the categories are). Relations between sample size and diversity have formerly been studied from an ecological (i.e., Kempton 1979, Krebs 1985, Magurran 1991) and a palynological point of view (Birks & Birks 1980, Rull 1987). Diversity measures can be applied to community species (specific diversity) as well as pollen types (typological diversity) (e.g., D'Antoni & Madanes 1986, Rull 1987, Moore et al. 1991). However, as we will discuss below, the interpretation of both is not exactly the same.

The Shannon-Weaver function *H'* (see Materials and Methods) is more sensitive to sample size than richness (Magurran 1991). As it can be seen, the *H'* versus sample-size curve reaches an asymptote earlier than the richness versus sample-size curve for the four sites (Fig. 2). Rull (1987) proposed to fix the minimum pollen sum at the diversity saturation point (DSP), where the curve becomes asymptotic and shows random fluctuations from that point onwards. With this method, our estimates of the minimum sample size were 150 grains (Fig. 2). It is important to remark that all the types incorporated beyond 150 grains are not abundant, always falling below 1%.

A different method is to compare the diversity estimates of different sample sizes using statistical techniques. Estimates calculated after repeated samples usually follow a normal distribution (Magurran 1991). Therefore, differences among them may be studied by means of an analysis of variance. We compared the calculated diversity for independent counts of 150, 300, and 600 grains for each site (Table II A). There were no significant differences either among counts or for the counts-sites interaction (*P*>0.05) (Table II B). Despite this, comparisons between the 150 vs >2700 grains-samples (Table III) show that the results depend on the site and so differences became significant for the NW and NE sites (*P*<0.001) and non-significant for the SW and SE sites (*P*>0.05). The dependence of the site is a consequence of the increase in richness as it can be seen when comparing the NE and SE sites.

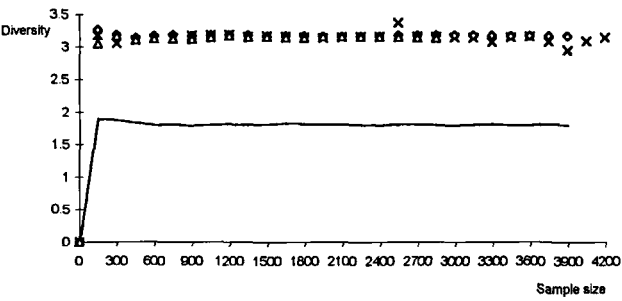


Fig. 2. Diversity (*H'*) versus sample size. X: site SW; —: site NW; Δ: site SE; ◇: site NE.

Table IIB. Analysis of variance.

SV—Source of variation; DF—Degrees of freedom; SS—Sum of squares; MS—Mean square; F—F-ratio; P—P-value.

SV	DF	SS	MS	F	P
Sample size	2	0.0011	0.0005	0.041	P=0.96
Sites	3	7.5936	2.5312	211.81	P<0.0001
Interaction	6	0.0664	0.0111	0.928	P=0.50
Error	12	0.1434	0.01195		

Comparing sites

Consistency of clustering using different sample sizes was analyzed. Cluster analysis was performed with both binary and quantitative coefficients (see Materials and Methods). Clusters based on 150 grains are different from those based on ≥ 2850 grains-samples when a binary coefficient is used (Fig. 3A, B). The clusters are sample-size dependent and spurious affinities may appear.

On the contrary, the clusters resulting from the quantitative coefficient are consistently similar (Fig. 3C, D). Both of them show that the NW site is different from the rest.

To test the classification obtained by the cluster analysis, two different methods were used.

Pielou's pooled quadrat method (see Materials and Methods) is a technique for estimating diversity. It provides an estimate of the population diversity from randomly accumulated samples and it can be used to estimate confidence limits (Magurran 1991). The observed values of r suggest that diversity variations do not depend on sample size ($P>0.05$ in all cases; Table IV). The estimates for the four sites showed that diversity does not show any significant differences among sites except for site NW.

As we saw in the previous section, analysis of variance of diversity shows that there are significant differences among sites ($P<0.05$) (Table II B). In fact, the diversity of the pollen spectrum of the NW site is significantly lower than the others'.

DISCUSSION

The airborne pollen from the studied agroecosystem

In the Balcarce Experimental Station, the survey revealed 49 plant species (Dadon & Madanes 1996) producing 49 pollen types, of which 43 were found in our traps. The presence of

6 pollen types from plant species not registered in this area was also recorded. Our actual performance could be considered good, but it involved an extremely high count (41,750 grains in total) and an experimental design with four simultaneous sampling sites. The complete spectrum including all pollen types present in the area was not registered in one site, even though the area was relatively small (7500 m²). The maximum number of types caught in one site only was 36 (84% of the total; Table I).

Pollen diversity, which we refer to as typological diversity to differentiate it from community specific diversity, is not the result of direct relationships among pollen types but of the inter-relationships among the pollen-producing plant populations and the effect of environmental factors. Therefore, the deposited pollen spectrum at a site is not the same as the pollen spectrum produced at the site, since the latter is modified by environmental factors that affect pollen dispersion, deposition, and preservation.

In fact, when comparing pollen spectra and the floral composition of the area, it was noteworthy that the cultivars (*Triticum*, *Zea* and *Helianthus*) were scarcely represented or virtually absent in most of the samples, in spite of being the dominant plants in the area. It is very well known that many important crop plants are only accidentally registered in the pollen count; this is due to zoophyllous pollination (as in the case of *Helianthus*) and artificial selection (in *Triticum*, *Zea* and *Helianthus*) as cultivars tend to be under-represented in pollen spectra (Faegri & Iversen 1992).

Taking into account that the gain of information is not linearly dependent on the counting effort, an acceptable characterization of site pollen for standard work might be obtained with a minimum sample size of 150 grains. Sample-size values obtained with this method are close to those recommended by others. For palaeoecological analysis, for instance Moore & Webb (1978) considered 150 grains to be an adequate size while Rull (1987) and Birks & Birks (1980) proposed 300–400 grains per sample. For AP analysis, 150 grains can be used to make inferences on the percentage of the dominant ($>2.5\%$) types (Booberg 1930, in Faegri & Iversen 1992) while 800–1000 (Bowman 1931) or 5,000 grains (Hafsten 1956) are necessary to make inferences about minor constituents. For surface soil samples, Hill (1996) considered that 250 grains is the lowest possible count which is imperative in sediments with a low pollen content, but a count of 1000 grains is needed for the most heterogeneous community of his study.

Table III. Number of pollen types, richness, evenness, and diversity (H') in the studied sites.

t—Student's test between typologic diversity estimates for each site; DF—degrees of freedom; P—P-value.

Site	SW		NW		SE		NE	
Sample size	150	3900	150	30950	150	3750	150	2700
Number of pollen types	19	30	10	26	16	28	14	28
Richness	4.24	4.90	3.32	4.70	4.00	4.90	3.80	4.80
Diversity (H')	3.17	3.12	1.89	2.04	3.20	3.15	3.04	3.12
t	1.43		3.13		2.24		3.28	
DF	163		154		157		174	
P	P>0.05		P<0.05		P>0.05		P<0.05	

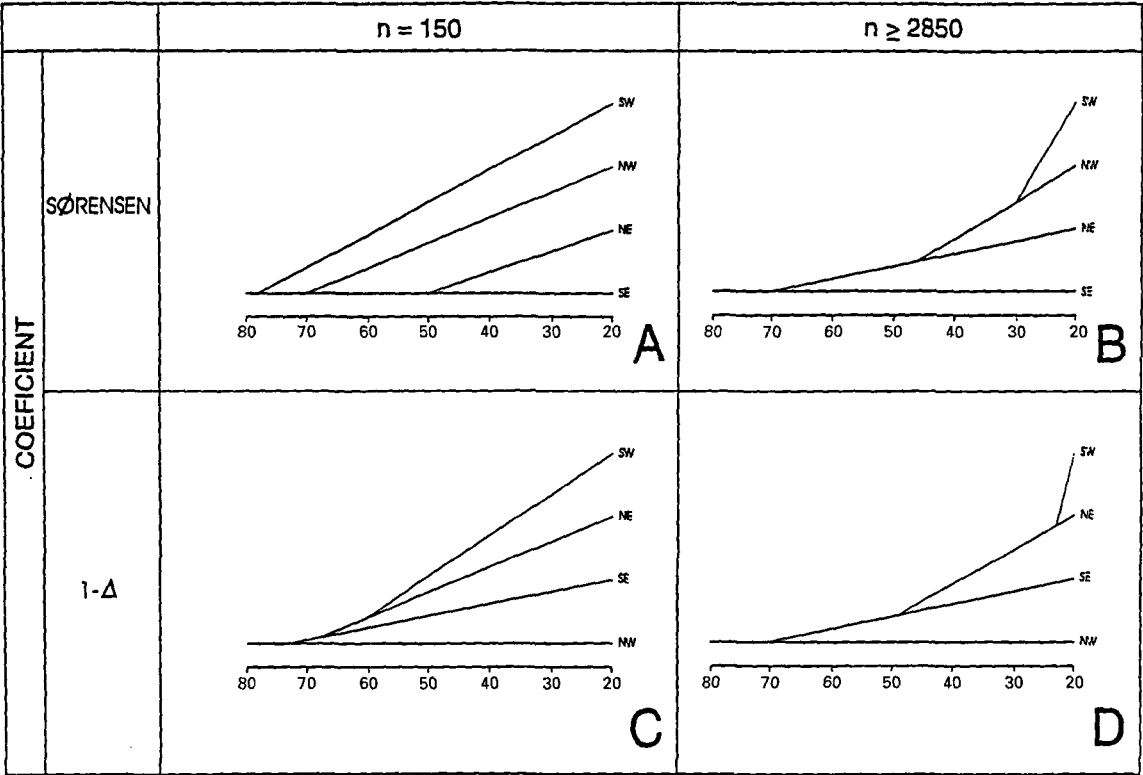


Fig. 3. Cluster analysis among sites for different sample sizes. A, B: Sørensen's (1948) coefficient; C, D: $1 - \Delta$ (Δ : Euclidean distance).

Table IV. Diversity (H'_{pop}) calculated by the pooled method (Pielou 1984).

r—Pearson's correlation coefficient (r) between sample size (from 150) and diversity; P—P-value.

Site	SW	NW	SE	NE
H'_{pop}	3.12	1.78	3.15	3.13
Standard deviation	0.087	0.180	0.100	0.115
r	0.29	0.27	0.34	0.37
P	P>0.05	P>0.05	P>0.05	P>0.05

When the sample size is fixed at 150, richness-dependent variables will be under-estimated and, consequently, diversity can be under-estimated too, but this bias is always due to rare types which overall are always extremely low. In fact, as the pollen count proceeded beyond 150 grains, there was an increment in the number of types (even up to a 100%), but the last incorporated types were always rare, their relative frequencies being both very low individually (always <1%) or collectively (up to 6%).

With 150 grain-samples it is possible to identify the dominant and subdominant types, which represent 70%–80% of the grains in the samples. In addition, similarity among sites are reliably estimated when appropriate quantitative coefficients are employed.

Assessment of the proposed method

Unless the purpose of investigation includes the search and identification of particular pollen types, a complete list

represents an excessive and unnecessary effort; taking this into account, many standard papers focus rather on the simultaneous evaluation of the species and their frequencies. In such works, synthetic indices are appropriate tools to evaluate the gain of information whilst increasing the sample size (Rull 1987).

The results obtained with the airborne pollen in the Balcarce Experimental Station showed that pollen richness could not be reliably estimated even with very large counts. In fact, counts as large as 3900 grains per site were able to detect only 61% of the types present in the area, thus under-estimating the actual total pollen richness. It is worth noting in light of these results that large counts should be made only when richness estimation and/or complete pollen type inventory are the objects of the research.

When the analysis of a large number of samples is implied and the aim of the investigation does not focus on the search and identification of a particular rare pollen type but on dominant species and/or assemblages as a whole, the minimum sample size should be considerably smaller. The minimum size chosen as shown here allows a reliable estimate of the major pollen components of the site, of the typologic diversity, and of the affinities among sites.

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