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EPIDERMOLOGY OF EUROPEAN FERNS

R.L.L. VIANE
Leerstoel voor Morfologie Systematiek en Ecologie van de Planten
9000 GENT

Summary.

A definition of epidermology, a historical background, a method to prepare fern leaflets and a computer program to make measurements are given. A more general formula to calculate cell dimensions for plants with a particular genome and polyploidisation factor "P" is presented. The importance of micro-characteristics in *Asplenium* (*Ceterach*) and in the *Dryopteris villarii* aggregate is shown, two keys are given.

Key words: Epidermology, *Asplenium, Dryopteris.*

Resumen.

Se presenta una definición y una revisión histórica de la epidermología, asi como un método para preparar pinnas de helechos y un programa informático para la realización de medidas. Se incluye, asimismo, una fórmula más general para el cálculo de loas dimensiones de las células para plantas con genomas particulares y poliploidización factor "P". Además se muestra la importancia de las microcaracterísticas en *Asplenium* (*Ceterach*) y en el agregado *Dryopyeris villarii.*

Palabras clave: Epidermología, *Asplenium, Dryopteris.*

1. DEFINITION.

Micromorphology is the study of the shape of structures too small to investigate scientifically with the naked eye or by simple optical means such as a hand lens. Depending on the structure or the object that one wants to study, a microscope or even a scanning electron microscope is needed, and preliminary preparation of the material is necessary. Any tissue (epidermis, parenchym, vascular strands, etc.), both from gametophytes and from sporophytes, can be studied. "Epidermology" is the discipline of micromorphology dealing only with epidermal structures; the one dealing with spores is usually called "palynology", but an acceptable correct name would also be "sporology".

2. MICROMORPHOLOGY, A SHORT HISTORICAL SKETCH.

In the sixteenth century, when microscopes became effectively used, spores and epidermal appendages were illustrated for the first time. In the first books with mainly fern illustrations (PLUMIER, 1703) small structures such as hairs, glands, sporangia and spores, were only rarely figured; HOFFMANN (1796) already distinguished species in *Gymnocarpium* by the presence (not by the form) or absence of glands. Many excellent figures of spores, sporangia, indusia, epidermal appendages (hairs and scales) and of venation patterns are found in the early eighteenth century books on ferns by HEDWIG (1799), SCHKUHR (1809), HOOKER and GREVILLE (1828-1831), KUNZE (1840-1851), BRACKENRIDGE (1854), etc. During this period microscopic structures became used in taxonomy. Thus, FÉE (1844-1866) in France was probably the first to use spore characteristics in fern classification; the middle European "school" of PRESL (1836) in Prague and of METTENIUS (1857-1859) in Leipzig also played an important role, yielding concepts such as anadromy and catadromy. METTENIUS divided several fern families into three groups: "Polystichoideae" with all 'last order' veins anadromous, "Phegopteroideae" with the lower part of the frond anadromous, but the upper part catadromous, and "Cyatheoideae" with mainly catadromous venation. MILDE, a student of METTENIUS, boiled plant parts for microscopical study (1867), and attached much importance to the indument and to venation patterns. Many later authors have (occasionally) used (the easily visible, or the presence/absence of) micro structures to distinguish taxa (CHRIST, 1899). Few comparative, micromorphological studies of entire genera or families have been done, but since the advent of the scanning electron microscope smaller plant structures get more attention again. To date, new cytological and other data should be regularly used to permit an improved evaluation of micro-characteristics for taxonomical purposes; modern micromorphology should be based on precisely measured and accurately illustrated structures, so that it yields repeatable results. Micromorphology is not just a scientific discipline on its own; it needs not only information from other fields, but it also feeds back and exchanges its own results and helps to induce new investigations elsewhere.

3. METHOD.

The following is a simple method to prepare dried leaf segments from herbarium material for microscopical observation. Segments should be as big as possible, and may be entire pinnae as long as these are mountable; too much fragmentation of the leaves results in a loss of information.

1. Boiling.

Depending on the thickness and toughness of the material, boiling lasts from one to several minutes or until the material has about the same consistency it had in the fresh state.

2. Clearing in sodium hydroxide and hypochlorite.

After boiling the material is put in a 2,5% NaOH solution for 24 hours, and is rinsed afterwards in running water during 15 minutes. Clearing is completed in a 50% solution of commercially available, concentrated (47/50) NaOCl; it takes from less than one minute, for thin leaflets, to possibly half an hour or even more for thick, coriaceous segments. This final bleaching should be followed carefully, as thin material tends to dissolve if it is kept too long in the NaOCl solution. Afterwards the objects are rinsed in running water for about one hour, then treated for 30 minutes in a 0,5% acetic acid (CH_3COOH) solution to remove remaining hypochlorite, and rinsed in running water again (one hour).

3. Staining.

The material is put in a fresh, dark reddish-purple, Ruthenium red [Ru2(OH)2Cl4.7NH3.3H2O] solution until it is stained medium to dark red. Depending on the consistency of the leaf, the dyeing will take from half to several hours, and should be checked regularly to avoid over staining. Overnight staining should also be avoided, or only exceptionally done in a more diluted solution, as the material easily becomes too dark. The coloured segments are then passed through the classical alcohol and xylene series, before mounting in Canada Balsam.

4. Why?

In epidermis preparations (prepared as indicated) several new (microscopic) characteristics become available for study (VIANE, 1988; RASBACH et al., 1989):

-the size, distribution and form of the epidermal cells;
-the size of guard cells, and stomatal types;
-indument types and sizes;
-the structure and the size of the indusium, and of its cells;
-the form and size of paraphyses;
-the form, structure, and condition of the sporangium;
-the presence and size of glands or hairs on the sporangium;
-the condition and size of the spores.

In comparative, morphological studies of uniseriate multicellular hairs on leaves, the following size characteristics (mean values) can be used (VIANE, 1986):

-Lt: the total length;
-Lc: the length of the upper cells;
-Wc: width of upper cells;
-Lt/Wc: the ratio of Lt to Wc;
-N: the number of cells in the hair.

Unicellular, stipitate or capitate, and clavate glands are typified (VIANE, 1986) by measuring:

-Lt: the total length (Lt=dA + Ls);
-dA: the apex diameter;
-dB: the diameter at the base;
-Ls: the length of the stalk;
-dA/Ls;
-dA/dB.

The thickness of the leaf, the presence of a hypodermis, the form and size of the cells and of their intercellular spaces can be studied in the mesophyll.

The venation pattern can be observed precisely if whole (or carefully cut) pinnae are mounted, since then the exact place of acroscopic and basiscopic branching is visible.

Micromorphology becomes an indispensable tool when important, old and incomplete, herbarium (type) material of closely related taxa (complexes or aggregates, which are otherwise best identified by studying their chromosome numbers), needs investigation.

The following alphabetical list is a selection of modern (after 1950) authors who used epidermological data in their (published) taxonomical work.

Author(s)	Subject or taxon	Year
Atkinson	-Gametophytes	-1973
Baayen and Hennipman	-Paraphyses (Polypodiaceae)	-1987
Barrington, Paris & Ranker	-Spore and stomata size	-1986
Bhambie and Madan	-Venation (Ophioglossaceae)	-1982
Bir and Trikha	-Scales (*Lepisorus*)	-1974
Chandra	-*Stenosemia* (Tectarioids)	-1983
Hauke	-*Equisetum* stem surface	-1979
Hennipman	-*Austrogramme*	-1975
Hennipman	-*Bolbitis*	-1977
Hennipman and Roos	-*Platycerium*	-1982
Hetterscheid and Hennipman	-Microsorioid Polypodiaceae	-1984
Holttum	-Various groups	-1959-1981
Holttum and Edwards	-*Dryopsis*	-1986
Hoshizaki	-Rhizome scales *Platycerium*	-1970
Hovenkamp	-*Pyrrosia*	-1986
Lellinger	-*Niphidium* scales	-1972
Lellinger	-*Campyloneurum*	-1988
Lommasson and Young	-Vascularization	-1971
Loyal	-*Pronephrium* & *Ampelopteris*	-1977
Mickel	-*Elaphoglossum*	-1987
Mickel and Atehortua	-*Elaphoglossum*	-1980
Mickel and Votava	-Leaf epidermis (*Marsilea*)	-1971
Moran	-*Olfersia*	-1986
Page	-Epidermis (SEM) in *Equisetum*	-1974
Payne and Peterson	-Leaf hypodermis	-1973
Pearson	-Rhizoids and root hairs	-1969
Phiri and Launert	-*Marsilea*	-1985
Rolleri	-*Lycopodium* epidermis	-1975, 1977
Roos	-Drynarioids	-1985
Sen and Hennipman	-stomata in Polypodiaceae s.s.	-1981
Sen and Sen	-*Oleandra* and *Nephrolepis*	-1973
Shing	-*Pyrrosia*	-1983
Smith	-*Thelypteris* subg. *Amauropelta*	-1974
Van Cotthem	-Typology of stomata	-1968-1973
Viane	-Several groups	-1975-1988
Wagner	-*Diellia*	-1952
Walker	-venation (*Polybotrya*)	-1985
Wiggins	-Cheilanthoids (Baja Californ.)	-1973

Excluding some more general investigations and studies on gametophytes and on spores and sporangia, it is striking that in only a few studies of European Pteropsida (numerical) epidermal characteristics were used:

Nardi, Rasbach and Reichstein	-*Cheilanthes*	-1979
Rasbach and Reichstein	-*Cheilanthes*	-1982
Vida, Mayor and Reichstein	-*Cheilanthes*	-1983
Rasbach, Reichstein and Schneller	-*Cheilanthes*	-1983
Viane	-*Dryopteris*	-1986, 1988

[Fortunately more are in progress, some were even represented during this congress (IBARS et al.; OTEO), while others (Strosse, 1987; Vervalcke, 1983) are published elsewhere].

One of the reasons is that only a few "difficult" complexes exist in the relatively poor European fern flora (*Asplenium, Cheilanthes, Cystopteris, Dryopteris*), that seem to warrant such a study. Another reason is the relative inacessibility of micro-characteristics (one must be prepared or able to use a microscope!) to field-botanists and amateurs, which severely limits their in keys and floras.

5. NUMERICAL DATA AND THE USE OF COMPUTERS.

Future micromorphological research will not be possible without the use of (personal) computers to make measurements, calculate mean values and other statistics, and to store data.

Instead of using the old, tedious, time consuming method of measuring with a calibrated ocular micrometer, preparations for micromorphological study can, much faster and more accurately, be investigated and measured using a microscope equipped with a drawing tube, connected to a digitising tablet and to a computer. To make this modern technique more widely available (avoiding a sophisticated and expensive program), a simple program written in GWBASIC is given below. "MM" consists of two parts, the first is a 'driver', steering the computer (any IBM compatible). In the second part, the distance between consecutive sets of two points is calculated, followed by some statistics. Since output is directed to a printer all lines with "LPRINT"-commands (460-530, 720, etc.), and lines 20, 36- must be eliminated if such a device is not available. The printer must be in "draft" mode, and if "letter quality" is desired line 360 must be adapted (see GWBASIC manuals).

```
10    'MM: Name of the program: is to be "loaded" at the start
20    X$=DATE$: LPRINT TAB(70) X$
30    CLS
40    PRINT"YELLOW—>DIGITISE"
50    PRINT"GREEN—>IMPORT"
60    PRINT"BLUE—>FINISH"
70    PRINT"WHITE—>ERASE": PRINT
80    PRINT"**IMPORTANT!** FIRST measurement must=***STANDARD LENGTH***"
90    'PART 1: DRIVER for digitising tablet ("SUMMAGRAPHICS") (by D&P Informatics,
      Brugge-Gent 1987)
100   OPEN"COM1: 9600,N,8,1,RS,DS" FOR OUTPUT AS # 2 LEN=1024
110   OUT ADR+1,1
120   PRINT #2,"S";CHR$(13)
130   PRINT#2,"i";CHR$(13)
140   CLOSE 2
150   ADR=&H3F8: DIM B(10): DIM XX(4096): DIM YY(4096): PO=0
160   OFX=0: OFY=0
170   OPEN"COM1: 9600,N,8,1,DS" FOR INPUT AS # 2 LEN=8092
180   OUT ADR+1,1
190   B=ASC(INPUT$(1,#2)): IF B=153 GOTO 250
200   IF B=217 THEN LOCATE 8,1: PRINT"OUT OF RANGE";CHR$(7): GOTO 190
210   IF B=155 THEN: GOTO 330
220   IF (B=156 AND C<>B) THEN C=B: PO=PO+1: XX(PO)=X: YY(PO)=Y: PRINT
      PO,X,Y
230   IF (B=154 AND C<>B) THEN LOCATE 9,1: C=B: PO=PO-1: PRINT
      PO,XX(PO),YY(PO)
```

```
240   GOTO 190
250   A$=INPUT$(8,#2): C=B
260   FOR T=1 TO 4: B(T)=ASC(MID$(A$,T,1)): NEXT T
270   X=(B(1)+B(2)*128)/20!-OFX: Y=(B(3)+B(4)*128)/20!-OFY
280   IF (X>300 OR Y>300) THEN GOTO 190
290   LOCATE 7,1: PRINT"X COORDINATE";: PRINT USING"####.#";X
300   LOCATE 8,1: PRINT"Y COORDINATE";: PRINT USING"####.#";Y
310   LOCATE 9,1: PRINT"                "
320   GOTO 190
330   CLOSE 2
340   PRINT
350   'PART 2: Data management and calculations (R. Viane)
355   'INPUT OF VOUCHER REFERENCES
360   WIDTH "lpt1:",130
370   LINE INPUT "NAME              =";A$
380   LINE INPUT "COLLECTOR + NR + HERB  =";B$
390   LINE INPUT "ORIGIN            =";C$
400   LINE INPUT "CHARACTER MEASURED    =";D$
410   LINE INPUT "REMARKS           =";R$
420   LINE INPUT "STANDARD LENGTH  (µm) =";STANDARD
430   WHILE ABS(STANDARD)<1
440   GOTO 420
450   WEND
460   IF LEN(A$)=0 AND LEN(B$)=0 AND LEN(C$)=0 AND LEN(D$)=0 AND LEN(R$)=0
       GOTO 530
470   IF LEN(A$)=0 THEN GOTO 480 ELSE LPRINT TAB(40) A$
480   IF LEN(B$)=0 THEN GOTO 490 ELSE LPRINT TAB(40) B$
490   IF LEN(C$)=0 THEN GOTO 500 ELSE LPRINT TAB(40) C$
500   IF LEN(D$)=0 THEN GOTO 510 ELSE LPRINT TAB(40) D$
510   IF LEN(R$)=0 THEN GOTO 520 ELSE LPRINT TAB(40) R$
520   LPRINT "  STANDARD LENGTH  = ";STANDARD: GOTO 550
530   LPRINT TAB(50) "IDEM"
535   'CALCULATION
540   OPEN "O",1,"POINTS"
550   PRINT#1,STANDARD;
560   FOR T=1 TO PO
570   PRINT#1,T;XX(T);YY(T);
580   NEXT T
590   RESET: CLS: SCALE=1: SUM=0:TL%=0
600   OPEN "I",1,"POINTS"
610   OPEN "A",3,"ALL"
620   OPEN "O",2,"DISTANCE"
630   INPUT#1,STANDARD: PRINT "standard length:"STANDARD
640   INPUT#1,I1,X1,Y1
650   INPUT#1,I2,X2,Y2
660   DX=X2-X1:DY=Y2-Y1
670   DISTANCE=SQR(DX*DX+DY*DY)*SCALE
680   IF I2=2 THEN SCALE=STANDARD/DISTANCE: GOTO 640
690   PRINT#2,DISTANCE;
700   PRINT#3,DISTANCE;
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```
710   PRINT DISTANCE "µm"
720   LPRINT CINT(DISTANCE) "-";
730   X1=X2: Y1=Y2
740   SUM=SUM+DISTANCE: TL%=TL%+1
750   IF NOT EOF(1) GOTO 640
760   MEAN=SUM/TL%: PRINT TAB(5) "mean      : "MEAN
770   LPRINT TAB(1) "Number of measurements :";TL%
780   LPRINT USING " * Mean      = ####.## µm";MEAN
790   RESET
800   OPEN "I",2,"DISTANCE"
810   DEV=0: MIN=1000: MAX=-1: DIM TIMES(500): TIMES(DISTANCE)=0
820   INPUT#2, DISTANCE
830   DISTANCE=CINT(DISTANCE)
840   DEV=(DISTANCE - MEAN)^2 + DEV
850   IF DISTANCE<MIN THEN MIN=DISTANCE
860   IF DISTANCE>MAX THEN MAX=DISTANCE
870   TIMES(DISTANCE)=TIMES(DISTANCE)+1
880   IF NOT EOF(2) GOTO 820
890   STDDEV=SQR(DEV/(TL%-1)): PRINT TAB(5) "standard deviation :  "STDDEV
900   LPRINT USING " * Standard deviation = ####.## µm";STDDEV
910   PRINT TAB(30)"min= "MIN;"  max= "MAX;
920   LPRINT"Range: " "("CINT(MIN")")"" - "CINT(MEAN-STDDEV)" -"CINT(MEAN)"
        -  "CINT(MEAN+STDDEV)" - ""("CINT(MAX")"
930   GOSUB 1340
940   RESET
950   PRINT
960   PRINT TAB(50) " **< ENTER >** = YES"
970   INPUT "save THIS LAST series of values (0=no)";E$
980   IF E$<>"0" THEN INPUT "give code ";F$ ELSE GOTO 1000
990   NAME "DISTANCE" AS F$: LPRINT: LPRINT "code ="F$
1000  INPUT "CALCULATION OF OVERALL MEAN, etc. (0=no)";G$
1010  IF G$="0" GOTO 1230
1020  OPEN "I",3,"ALL"
1030  SUMM=0: MEANN=0: N%=0: DEV=0: MIN=1000: MAX=-1: TIMES(DISTANCE)=0
1040  INPUT#3,DISTANCE
1050  SUMM=SUMM+DISTANCE: N%=N%+1:
1060  IF DISTANCE<MIN THEN MIN=DISTANCE
1070  IF DISTANCE>MAX THEN MAX=DISTANCE
1080  MEANN=SUMM/N%
1090  DEV=(DISTANCE-MEANN)^2+DEV
1100  TIMES(DISTANCE)=TIMES(DISTANCE)+1
1110  IF NOT EOF(3) GOTO 1040
1120  PRINT TAB(5) "overall mean :"MEANN
1130  PRINT TAB(5) "total number of measurements ="N%: LPRINT: LPRINT
1140  LPRINT TAB(50) "TOTAL NUMBER of MEASUREMENTS = "N%
1150  LPRINT TAB(50) USING " * OVERALL MEAN =####.##µm";MEANN
1160  SD=SQR(DEV/(N%-1)): PRINT TAB(1) "standard deviation= "SD
1170  LPRINT TAB(50) USING " * STANDARD DEVIATION= ####.##µm";SD
1180  PRINT "min= "MIN;"   max= "MAX
1190  LPRINT TAB(1) "FOR ALL:  ("CINT(MIN")"  "-"CINT(MEANN-SD)"-
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```
      "CINT(MEANN)"-"CINT(MEANN+SD)"-""("CINT(MAX)")"
1200  GOSUB 1340
1210  RESET: PRINT
1220  PRINT TAB(50) " **< ENTER >** = YES "
1230  INPUT "Are you finished with ALL measurements of this SERIES (0=no)";H$
1240  IF H$<>"0" THEN INPUT "save ALL-values (0=no,)";ANT$ ELSE GOTO1300
1250  IF ANT$<>"0" THEN INPUT "new code for ALL-file=";CODE$ ELSE GOTO 1280
1260  LPRINT: LPRINT TAB(50) "Code of file with above values:"CODE$
1270  NAME "ALL" AS CODE$: LPRINT: GOTO 1300
1280  INPUT "dispose of ALL-file (0=no)";DIS$
1290  IF DIS$<>"0" THEN KILL "ALL" ELSE GOTO 1300
1300  INPUT "RUN THIS PROGRAM AGAIN (0=no;)";K$
1310  IF K$<>"0" THEN RUN
1320  ELSE GOTO 1330
1330  CLS: END
1340  PRINT: FOR M%=MIN TO MAX
1350  IF TIMES(M%)<>0 THEN PRINT USING "times(#):";M%;
1355  PRINT STRING$ (TIMES (M%), ".")
1360  NEXT M%
1370  PRINT TAB(50) " **< ENTER >** = YES "
1380  INPUT "print histogram (0=no)";N$
1390  IF N$="0" GOTO 940
1400  FOR O%=MIN TO MAX
1410  IF TIMES(O%)<>0 THEN LPRINT USING "times(#):";O%;
1415  LPRINT STRING$(TIMES(O%),"*")
1420  NEXT O%
1430  RETURN
1440  END
```

The above program can be used to measure lengths, widths, diameters etc. of any microscopial or macroscopical structure. For accurate microscopical measurements precise scaling needs to be done first, a chart with "standard units" (e.g. of 10 or 100 µm) for each setting of the microscope should result. When the program is started such "standard unit", corresponding to the microscopic enlargement used, must be "measured" first. At least a "four button cursor" must be connected to the digitizer.

The values of means and standard deviations given here are all rounded to the nearest digit, statistically this is not always correct, but I prefer this because the precision of each measurement is 0.5 µm using a digitiser and computer, and only 2-4 µm by more classical means; using decimals would then given an over stressed impression of more accuracy.

6. THE POLYPLOIDY FACTOR.

Within a species that consists of diploid and (several) (auto)polyploid subspecies, but also within groups of related (allopolyploid) species, cell dimensions, such as stomatal and exospore length, are highly correlated with the ploidy level; when cytological methods cannot be used they are among the most important characteristics to give a reliable estimation of the ploidy. The

correlation between cell dimensions and ploidy must be checked in each group (complex, genus...) investigated, using cytologically studied reference material (see also a.o. MORAN, 1982, and BARRINGTON et al., 1986 for a review).

When two (diploid) taxa (e.g. with genome formula AA and BB) hybridise, and their hybrid (AB) by polyploidisation forms a fertile allo(tetra)ploid plant (AABB), then the cell dimensions of this plant (AABB) are not exactly medial between those of the parental taxa (AA and BB), but are slightly larger by a ratio called the polyploidy factor "P" [VIANE, (1986), the 'value' of cell dimensions is indicated between straight lines (|)].

$$|AABB| = \frac{|AA| + |BB|}{2} \times P \qquad [1]$$

Thus, by definition, the value of "P" (for a particular characteristic and a particular taxon) is the ratio of two times the cell dimensions of a n-ploid taxon to the sum of the cell dimensions of its n/2-ploid ancestor(s):

$$P = 2\frac{|AABB|}{|AA| + |BB|}$$

P has values between 1.01 and 1.40. If doubling of the genome also yields doubling of the cell volume, BARRINGTON et al. (1986) point out that then in more or less spherical structures such as spores and stomata, a doubling of the cell volume corresponds to an increase of the diameter (=length) with a 'constant' of 1.26 (=P-factor). Evidently only in few cases such a 'theoretical' P-value is correct (see also the examples given below), but it is a good (average) approximate value, when "P" can not be calculated.

For autotetraploids P is simply the ratio of the cell dimensions of the autotetraploid taxon to the cell dimensions of its diploid ancestor:

$$|AAAA| = \frac{|AA| + |AA|}{2} \times P$$

or $$|AAAA| = |AA| \times P \qquad [2]$$

and thus $$P = \frac{|AAAA|}{|AA|}$$

When we use a different notation, and put AA=2A and AAAA=2A2A=4A then:

$|4A| = |2A| \times P$; and similarly for a diploid:

$$|AA| = |A| \times P \quad \text{or} \quad |2A| = |A| \times P \qquad [3]$$

and also: $$|AAAA| = |2A| \times P$$

$$= |A| \times P^2 \quad \text{or} \quad |4A| = |A| \times P^2 \qquad [4]$$

Where |A| stands for the value (of cell length, width, etc.) in the 'haploid plant' with genome A; as long as a diploid (AA) and an autotetraploid (AAAA) are known and if we accept that P keeps the same value (from A to AA as from AA to AAAA), this value can be calculated.

The general formula for an auto-n-ploid taxon, with known P and known |A|, is then:

$$|nA| = |A| \times P^{\log_2 n} \qquad [5]$$

or also

$$|nA| = |AA| \times P^{(\log_2 n)-1}$$

If P is unknown, it can be calculated (for such an autopolyploid!), by the general formula:

$$P = \sqrt[\log_2 z]{\frac{|zA|}{|A|}} \qquad \text{or} \qquad P = \sqrt[(\log_2 z)-1]{\frac{|zA|}{|AA|}}$$

After some calculations the following general formula, can be deduced:

$$|iA...nX| = \frac{i|A| +...+ n|X|}{i +...+ n} \left(\frac{iP_A +...+nP_X}{i +...+ n}\right)^{\log_2 (i +...+ n)} \qquad [6]$$

where 'i' to 'n', are the number of genomes (A to X) present; P_A to P_X are the P factors (for genomes A to X respectively), if these P-values are unknown and if almost spherical to elliptical structures are studied, P=1.26 may be used. This formula is different from BARRINGTON'S (1986) in the use of the logarithm (with base 2!) for the power of "P".

That this is a general formula can be shown by the following simple (but common) cases, where [6] converts to the formulas already deduced, thus:

1. For AB, the hybrid between AA and BB

$$|AB| = \frac{|A| + |B|}{2} \times \frac{(P_A + P_B)^{\log_2 2}}{2} \quad (\text{with } \log_2 2 = 1)$$

$$= \frac{|A| + |B|}{2} \times \frac{P_A + P_B)}{2} \qquad [7]$$

for A=B and $P_A=P_B=P$, this gives formula [3] again.

If $|A| = \frac{|AA|}{p}$ and $|B| = \frac{|BB|}{p}$, then: $|AB| = \frac{|AA| + |BB|}{p}$ thus [7] also shows that cell dimensions in a hybrid are intermediate (average) between the dimensions of their parents (if $P_A=P_B$!), as is often found in nature.

2. For an allotetraploid AABB, with P_A P_B.

$|AABB| = |2A2B|$

$$= \frac{2|A| + 2|B|}{2+2} \times \left(\frac{2P_A + 2P_B}{2+2}\right)^{\log_2 (2+2)} \qquad [8]$$

$$= \frac{|A| + |B|}{2} \times \left(\frac{P_A + P_B}{2}\right)^2 \qquad \text{and if } P_A + P_B = 2P$$

$$= \frac{|A| + |B|}{2} \times P^2 \qquad [9]$$

This is the same as formula [1], since substituting in [1] of |AA| or |2A| and |BB| or |2B| with [3] gives:

$$[1]=\frac{|A|P+|B|P}{2} \times P=[9]$$

3. For autopolyploids, the (general) autopolyploid formula [5] is immediately evident as substitution of i+...+(n-1)=n, A=...=X=A, and of $P_A=...=P_X=P$ shows.

The general formula [6] can be used in predicting cell dimensions of unknown polyploids and hybrids with any genome combination, as long as the dimensions of the ancestral genomes and the "P"-factors, are known or can be calculated. Predictions will be closer the more polyploid series are available for study, and the less cell dimensions are influenced by the environment (stomata, exospore).

The P-factor, together with cell dimensions can also be used to test relationships (BARRINGTON et al., 1986; VIANE, 1986); when P_x is smaller than 1.00 or bigger than 1.50 for several characteristics, the proposed ancestry of the taxa studied should be re-investigated.

7.RESULTS OF MICROMORPHOLOGICAL STUDIES IN *ASPLENIUM* (INCL. *CETERACH*).

The following is an example, taken from a recent study (H. & K. RASBACH & VIANE, *1989*) of the type of *Asplenium* x *badense* (Meyer) Jermy, the putative hybrid of *A. ceterach* L. and *A. ruta-muraria* L., which is compared to *A. aureum* Cav. and other related ferns. In table I, the number of measurements (n), the mean length of the stomata (sto-length) and of the exospore (exo-length), their standard deviation (sd) in µm, and the polyploidy factor (P), are given:

TAXON	PLOIDY	n	STO-LENGTH	sd	P-factor
A. aureum s.l.	4n	50	41	3	1.34
	6n	109	45	5	
	8n	70	55	6	
A. ceterach	2n	204	36	5	1.14
	4n	157	41	4	
x A. badense	4n?	104	56	4	
A. ruta-muraria	2n	120	40	4	1.25
	4n	158	50	6	

TAXON	PLOIDY	n	EXO-LENGTH	sd	P-factor
A. aureum s.l.	4n	81	37	2	1.24
	6n	73	38	3	
	8n	64	46	4	
A. ceterach	2n	153	31	3	1.22
	4n	100	38	3	
x A. badense	4n?	142	45	4	
A. ruta-muraria	2n	118	39	2	1.15
	4n	294	45	4	

TABLE. I

Using the appropriate P-values (see above), some more guard cell and exospore lengths have been calculated (=followed by "!") for interesting genome combinations discussed further below. (Au=of 1 genome of *A. aureum*, Ce=of 1 genome of *A. ceterach*, and Ru=of 1 genome of *A. ruta-muraria*; original dimensions, from table I, are in bold and underlined).

Below the diagonal, in the lower left half, are the guard cell lengths; above the diagonal are exospore lengths! Each cell represents one genome combination, which can be found by adding the

genome	EXO	Au	2Au	4Au	8Au	Ce	2Ce	4Ce	Ru	2Ru	4Ru
STO	* * *	24!	30!	37	46	25!	31	38	34!	39	45
Au	23!	* * *				30!					
2Au	31!	37!	* * *	42! 38			37!	42!			■
4Au	41		49! 45	* * *			42!	46!			
8Au	55				* * *						
Ce	32!	34!	38!			* * *			(35)		
2Ce	36	39!	42!	49!		39!	* * *			41!	
4Ce	41		47!	52!		43!	45!	* 48! * 47! *			49!
Ru	32!					38!			* * *		
2Ru	40						46!			* * *	
4Ru	50							55!			* * *

TABLE. II.

combinations shown for that particular row and column. Thus in the cell marked " ■ ", the exospore would be given for genome 2Au+4Ru [a plant that could originate from a 3-ploid hybrid between diploid "aureum" and tetraploid "ruta-muraria", followed by chromosome doubling (2Au x 4Ru → AuRuRu → 2Au4Ru)].

Using the appropiate Pg.cell and Pexosp., we have calculated that the (probably fertile) allotetraploid fern (CeCeRuRu) originating (after chromosome doubling) from the hybrid (CeRu) between diploid *A. ruta-muraria* ssp. *dolomiticum* (RuRu) and diploid *A. ceterach* ssp. *bivalens* (CeCe) (both not recorded for the type locality of *A. badense*!) would have guard cells c.46 µm and exospores c.41 µm long. The (probably sterile) tetraploid hybrid [CeCe'RuRu'=*A. x badense* sensu

Meyer (1957)], that originates from a direct cross between tetraploid *A. ruta-muraria* (RuRuRu'Ru') and tetraploid *A. ceterach* (CeCeCe'Ce') (both present at the locus classicus), would have guard cells also c.46 µm long, but should have mostly aborted spores; the allo-octoploid (CeCeCe'Ce'RuRuRu'Ru') that would originate (after polyploidisation) from this sterile tetraploid hybrid, would have guard cells 55 µm and exospores 49 µm long. Since the calculated cell dimensions, for the allotetraploid (CeCe'RuRu') are significantly smaller than what we actually find in the plant described as *A. x badense*, this fern can not have the genome that was postulated by Meyer. From its cell dimensions, it can then either be a (partly fertile) auto-octoploid *A. ceterach* or also a (party fertile) allo-octoploid hybrid ("*x Asplenioceterach*"), however since non of the other character is intermediate (contrary to Meyers indications, 1957) between those of its presumed parents, *A. x badense* can not be the hybrid between *A. ceterach* and *A. ruta-muraria*! Its overall morphology shows that it belongs the *A. ceterach* group, micromorphology not only confirms this, but strongly indicates that the plant could well be a hitherto unknown octoploid cytotype of *A. ceterach.*

The origin of *A. aureum* and its status still require further study (see MANTON et al., 1986); for simplicity and in anticipation of more evidence in the future, I have considered *A. aureum* ssp. *parvifolium* an auto-octoploid here, (if it is an "allo", then the true auto-octoploid should have guard cells c.58 µm long, with P=1.43 and lAul=20 µm; and its exospore would have to be 47 µm long, with P=1.28 and lAul=23 µm). The cell dimensions of spores and stomates of the hexaploid cytotype are smaller than those calculated (followed by "!" in table II), this is also true when ssp. *parvifolium* is treated as an allopolyploid! Hitherto no diploid cytotype of *A. aureum* has been described; if it ever existed, it must now be either extremely rare or extinct. If *A. ceterach* and *A. aureum* are considered distinct species there are several possible ways of origin for the hexaploid; three possible genome formulas are:

1. AuAuAuAuAuAu or 6Au for an 'auto'polyploid, either via polyploidisation (-»») in a triploid (2Au x 4Au -» 3Au -»» 6Au) or via a direct cross (4Au x 8Au -» 6Au), with guard cells 49 and exospore 42 µm long (table II).

2. AuAuAuAuCeCe or 4Au2Ce, also via polyploidisation of a triploid hybrid (4Au x 2Ce -» 2AuCe -»» 4Au2Ce) or as the result of a direct cross (8Au x 4Ce -» 4Au2Ce), with guard cells 49 and exospore 42 µm long (table II).

3. AuAuCeCeCeCe or 2AuCe, again either via polyploidisation (2Au x 4CeCe -»Au2Ce -»» 2Au4Ce) or direct (4Au x 8Ce -» 2Au4Ce), with guard cells 47 and exospore 42 µm long (table II). Obviously, cell dimensions are of little help here, but they do show that the three genome formulas have equal potential.

8.MICROMORPHOLOGICAL STUDIES IN *DRYOPTERIS.*

A. *Dryopteris x gomerica* in Spain.

When, in 1986, *D. x gomerica* was discovered in Asturias (VIANE, 1988), its indument was first studied and distinguished from that of its ancestors: *D. aemula* and *D. guanchica.* Later cytological study confirmed these micromorphological findings.

B. The *D. pallida-villarii* group.

This group consists of a complex of at least four related taxa: two ancestral diploids and

two (allo)tetraploids. Its taxonomy has recently been studied by several authors (a.o. FRASER-JENKINS, 1977, 1982, 1986; FRASER-JENKINS & REICHSTEIN, 1975), but here only the results of an epidermological study of the following 'mediterranean' taxa are given (genome formula between brackets):

1. ***D. pallida*** (Bory) Fomin, a diploid (PP) with four subspecies:

ssp. *balearica* (Litard.) Fraser-Jenkins	(PbPb)
ssp. *libanotica* (Rosenst.) Fraser-Jenkins	(PlPl)
ssp. *pallida*	(PpPp)
ssp. *raddeana* (Fomin) Fraser-Jenkins	(PrPr)

2. ***D. submontana*** (Fraser-Jenkins & Jermy) Fraser-Jenkins, an (allo)-tetraploid (PPVV) that (probably) originated from a hybrid (*D. vidae* Fraser-Jenkins & Gibby) between *D. pallida* (PP) and *D. villarii* (VV);

3. ***D. tyrrhena*** Fraser-Jenkins & Reichstein, an allotetraploid (OOPP), assumed to have formed originally from a diploid hybrid between *D. oreades* Fomin (OO) and *D. pallida* (PP);

4. ***D. villarsii*** (Bell.) Woynar ex Schinz & Thell., an (alpine) diploid (VV);

5. ***D. oreades*** Fomin, an ancestral diploid (OO).

The taxa (1-4) have often been confused in the past; the diploids, (easily) distinguished on gross morphology, are also ecologically separated. The tetraploids are (sometimes) differentiated with great difficulty (even from the diploids); they are so similar to each other that identification of (poor) specimens is almost impossible, even for specialists. The taxonomy of the group is not yet clear: in a recent publication about the genus *Dryopteris*, FRASER-JENKINS (1986) puts *D. pallida* and *D. submontana* (!) in *D.* section *Pallidae*, but *D. tyrrhena* (!), *D. villarii, D. oreades*, etc. in section *Dryopteris*. If this classification is adopted it should be modified first so that both *D. submontana* and *D. tyrrhena* are in one (intermediate) section of their own.

For this study glands have been measured on the dorsal side of the lamina. I have found no significant size differences among glands from above various parts of the vascular tissue (costa, costula, veins), nor between these and those from the non vascularised parts of the lamina. Glands on indusia are usually distinctly smaller and their dimensions are not used here. The results are presented in the tables below. The values given are the mean and the standard deviation, all in µm (abbreviations see under 4).

1. Subspecific taxa in *D. pallida.*

If these "MEAN"-values of micro-characteristics are closest to the original "ancestral" values that existed in the ancestral stock from which the subspecies evolved, then the subspecies with values closest to those of the above "MEAN", might also be closest to this ancestor. Therefor I consider the most widespread subspecies: ssp. *pallida*, that to date has values closest to the "MEAN", to represent the most original condition in the group. This is more clear when the percent change (%), the absolute cumulated change ( ) and its mean "M", and the number of changes "N", in relation to the "MEAN" are given (TABLE 2). The coefficient of variation "CV" (CV=standard deviation/mean x 100; SOKAL & ROHLF, 1980), enabling us to compare the relative amounts of variation of the characters, [but see LEWONTIN (1966) and SOKAL & BRAUMANN (1980) for a statistical discussion!], is included.

TAXON	Lt	dA	dB	Ls	dA/Ls	dA/dB	Lstom	Lexo
D. pallida ssp. balearica	70±10	27±3	17±3	43±7	0.6	1.6	38±4	34±3
ssp. libanotica	65±9	34±6	17±3	31±9	1.1	2.0	41±5	32±2
inter ssp. libanotica & pallida	66±8	32±5	17±2	34±8	0.9	1.9	39±2	33±2
ssp. pallida	73±8	32±5	18±3	41±8	0.8	1.8	39±4	34±2
ssp. raddeana	83±10	32±4	19±2	51±9	0.6	1.7	42±4	35±2
"MEAN"	71+10	31+5	18+3	40+10	0.8	1.8	40+4	34+2

TABLE 1. Micromorphological values in subspecies of *D. pallida*.

TAXON	Lt	dA	dB	Ls	Lstom	Lexo	Σ&M	N
D. pallida ssp. balearica	-1% CV:14	-13% CV:11	-6% CV:17	+7.5% CV:16	-5% CV:10	0% CV:9	Σ32.5 5.4%	5
ssp. libanotica	-8% CV:14	+10% CV:18	-6% CV:18	-22.5% CV:29	+2.5% CV:12	-6% CV:6	Σ55 9.2%	6
ssp. pallida	+3% CV:11	+3% CV:16	0% CV:17	+2.5% CV:20	-2.5% CV:10	0% CV:6	Σ11 1.8%	4
ssp. raddeana	+17% CV:12	+3% CV:13	+6% CV:13	+27.5% CV:18	+5% CV:10	+3% CV:6	Σ61.5 10.3%	6

TABLE 2. Changes in relation to the "MEAN".

Just as in many other fern groups, "CV" shows that exospore and stomata length are less variable than the other characteristics. That the dimensions vary least in ssp. *raddeana* but most in ssp. *libanotica*, can be explained in several ways:

1. of ssp. *raddeana* only one specimen has been studied, and the "CV" of a single plant is usually less then the "CV" of the whole population and of the species (many populations)! This is contrary to some statements in the recent literature, but there the "wrong standard deviation" (the standard error) had repeatedly been used. Study of more specimens of this rather rare plant should be done.

2. The lower "CV" of ssp. *raddeana* could also be due to more stable ecological conditions in its (restricted) habitat(s) compared to possibly more diverse conditions at the localities of the more widespread ssp. *pallida*. An ecological study should be made to confirm this.

3. However, the characters of ssp. *raddeana* might be more "stabilised" due to its longer and wider isolation from the other taxa; they may be more variable in ssp. *libanotica* because this might be a more recent, still diversifying, and less "isolated" subspecies (intermediates between ssp. *libanotica* and ssp. *pallida* exist, see above, and FRASER-JENKINS, 1977.

If ssp. *pallida* is considered to be most similar to the ancestral stock, micromorphology seems to shows that *D. pallida* s.l. is diverging into three lines. *D. pallida* ssp. *pallida* and ssp. *balearica* are micromorphologically very close (according to FRASER-JENKINS, 1982, intermediate plants exist). Thus it seems as if in the western part of the range, *D. pallida* ssp. *balearica* became isolated and developed more densely glandular fronds and glands with smaller apex. At the eastern limits of the range two microscopically more diverse taxa developed. *D. pallida* ssp. *libanotica* lost the glands on its indusium, its gland length decreased while the apex enlarged, it developed more marginal sori, and smaller spores evolved. Fertile, intermediate plants between ssp. *libanotica* and ssp. *pallida* exist in Turkey. The fourth, and geographically most isolated, taxon *D. pallida* ssp. *raddeana*, is microscopically as different from ssp. *pallida* as is ssp. *libanotica*; its glands and other cells are bigger (acc. to FRASER-JENKINS this is a bigger plant on the whole).

Cytological, chemical, and the present epidermological study show that none of the taxa within *D. pallida* does deserve specific status (NARDI, 1976); it would even be better to recognise them only at the varietal level.

Key (microscopical) to the subspecies (using mean values in µm).

1.	dA<30	ssp. ***balearica***
	dA>30	2
2.	Ls<35, indusium eglandular	ssp. ***libanotica***
	35<Ls<45, indusium glandular	ssp. ***pallida***
	Ls>45, indusium glandular	ssp. ***raddeana***

2. Indument differences between the species in the *D. villarii* complex.

Data of a fifth taxon, the diploid *D. oreades* Fomin, one of the putative ancestors of *D. tyrrhena* are included.

The present epidermal study shows only minor differences between the diploids: *D. pallida* and *D. villarsii*; these species might be more closely related than recent cytological investigations seem to suggest (FRASER-JENKINS & GIBBY, 1980). One of the main microscopical differences (next to stomatal and exospore size) not yet mentioned, is the absence of a gland on the relatively

TAXON	Lt	dA	dB	Ls	dA/Ls	dA/dB	Lstom	Lexo
D. oreades	64±9	35±5	16±3	29±4	1.2	2.2	47±4	35±3
D. pallida	71±10	31±5	18±2	40±10	0.8	1.7	40±4	34±2
D. submontana	73±11	35±4	20±2	38±10	0.9	1.8	53±4	40±3
D. tyrrhena	90±9	40±4	18±2	50±9	0.8	2.2	50±4	38±3
D. villarii	72±9	34±4	18±2	38±9	0.9	1.9	47±4	37±2

TABLE III.

short-sporangium stalk of *D. villarsii*. Both taxa probably evolved from the same ancestral stock as *D. oreades*. Considering the differences both in gross morphology, micromorphology, ecology and cytology they are best treated as two separate species.

Though they have only one parent in common, the tetraploids *D. submontana* (PPVV) and *D. tyrrhena* (OOPP) are very similar both in gross morphology (see also RASBACH el al., 1982) and in their chemical composition. This might also be another indication that *D. oreades* (OO) is (distantly) related to *D. villarii* (VV) as suggested by FRASER-JENKINS (1986). Microscopically *D. submontana* and *D. tyrrhena* can be distinguished by their different gland dimensions; the sporangium stalk is always glandular in *D. tyrrhena* but in *D. submontana* stalks without gland may also be present.

Key (microscopical) to the species (using mean values in µm).

1. Lstom smaller than 50 µm 2
   Lstom equal or more than 50 µm 4
2. Lstom<45, sporangium stalk glandular 3
   Lstom>45, sporangium stalk not glandular ..... ***D. villarsii***
3. Lamina glabrous to sparsely glandular, indusium with only marginal glands, Ls<35 ..... ***D. oreades***
   Lamina and indusium always glandular (excl. ssp. *libanotica*), Ls>35 ..... ***D. pallida***
4. Ls<45, Lt<80, some sporangium stalks without gland ..... ***D. submontana***
   Ls>45, Lt>80, sporangium stalk always glandular ..... ***D. tyrrhena***

Micromorphology is at its best when other techniques fail to give answers, and when it can exchange information with a.o. general morphology, cytology, chemistry, ecology and geography. Thus, one result of the present study is that the range of *D. tyrrhena* may have to be enlarged, since a specimen (Wolff sn.) deposited in Brussels (BR) under *D. villarsii* seems to be *D. tyrrhena*; according to its label the specimen was collected in northern Yugoslavia: "Fiume, Mte. Magiore (1340 m), Istria", an area were the species has not been recorded before. Further investigations should be undertaken by local botanists. A rather poor, infertile specimen collected by Font Quer (Nº 72556 - BC) in NE Spain, (Vall de la Monrela, C. Horta de Tarragona) has stomata 50±4 µm, glands with Lt=67, dA=30, dB=15 and Ls=37 µm long; which key it out to *D. submontana* (as suggested by L. Sáez, on one label), and not to *D. pallida* (as shown on another label). This locality is not mentioned in FRASER-JENKINS (1982) nor in the Flora Ibérica (CASTROVIEJO et al., 1986).

A complete list of studied specimens is available from the author.

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