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# Quantifying Meiosis: Use of the Fractal Dimension, $D_f$ , to Describe and Predict Prophase I Substages and Metaphase I

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## 1. Introduction

### 1.1 Meiosis – Plants do it!

Plants undergo meiosis as a part of their life cycle, a fact that is sometimes forgotten and often misunderstood. Plants have a sporic life cycle wherein the living organism alternates between haploid and diploid states. Consequently, this cycle is also known as the “alternation of generations” (Gilbert, 2000). The generalization that states, “meiosis produces haploid gametes” is not technically true with respect to the intrepid members of Kingdom Plantae. Unlike the case in animal cells, the diploid plant (the sporophyte or “spore-producing plant”) possesses “germ-line” cells (sporocytes) that undergo the reductive division of meiosis I followed by meiosis II to produce haploid spores. The spores then undergo mitosis, growing into a haploid “organism”, which, in the flowering plants, is a small “gametophyte” (gamete-producing plant) housed within the confines of the original spore. In flowering plants, the female gametophyte (megagametophyte) held within the female spore (megaspore) inside the flower produces the egg, while the male gametophyte (microgametophyte) develops within the male spore (microspore). The microspore is better known as an immature pollen grain, and upon maturity, formation of the microgametophyte within the pollen effectively generates sperm. Ultimately, the pollen containing the sperm is released to find or “pollinate” the flower containing the egg, and two sperm are released from the pollen, one of which fertilizes the egg, forming a zygote and thus the next diploid sporophyte (Gilbert, 2000). The intricacies of flowering plant reproduction will not be described here: suffice it to say that plant sex is not quite as simple as the preceding story (for example, in flowering plants, a second fertilization event occurs, which forms a nutritive tissue called the endosperm). While leading to spores rather than gametes, meiosis the process in plant cells is *de facto* similar to that in animals.

## 1.2 Meiosis – A discrete continuum?

Meiosis, regardless of the organism in which it is occurring, is most often described as a series of discrete stages, even though it is really a continuum of events that flow from one to the next in a typically seamless and apparently effortless *pas de deux*. As will be described elsewhere in this book in more detail, the two meiotic divisions, meiosis I and meiosis II, are each conveniently subdivided into the familiar stages of prophase, metaphase, anaphase, and telophase, I and II, respectively. The most prolonged and intricate stage of meiosis is prophase I, which is highly regulated and concludes with the regimented alignment of homologous pairs along the equator at metaphase I (Alberts *et al.*, 2002).

During prophase I, replicated homologous pairs find each other in a process called “synapsis”; the synapsed homologues are often referred to as bivalents or tetrads, as a bivalent/tetrad has two chromosomes and four chromatids (Snustad & Simmons, 2008). DNA is exchanged between homologous chromosomes, usually resulting in chromosomal crossover. As prophase I is so protracted and complicated, it is usually described in five substages: leptotene, zygotene, pachytene, diplotene, and diakinesis.

The first stage of prophase I is the leptotene stage, also known as leptonema, from Greek words meaning "thin threads" (Snustad & Simmons, 2008), and is typically very short. Individual chromosomes—each consisting of two sister chromatids—change from the diffuse state they exist in during the cell's period of growth and gene expression, and condense into visible strands within the nucleus. However, the two sister chromatids are tightly bound to and hence indistinguishable from one another.

The zygotene stage, also known as zygonema, from Greek words meaning "paired threads", occurs as the chromosomes approximately line up with each other into homologous chromosome pairs in the initiation of synapsis (Snustad & Simmons, 2008). This is also called the “bouquet” stage because of the way the telomeres (chromosome “ends”) cluster at one end of the nucleus. Pairing is brought about by a zipper like fashion and may start at any point along the chromosomes. While the initial alignment is approximate, the pairing is highly specific and exact, and complete by the end of zygotene.

The pachytene stage, also known as pachynema, from Greek words meaning "thick threads", is the stage when chromosomal crossover (“crossing over”) occurs (Snustad & Simmons, 2008). In this process, nonsister chromatids of homologous chromosomes randomly exchange segments over regions of homology. The actual act of crossing over is not perceivable through the microscope, however, and pachytene is physically similar to zygotene, although the chromosomes are more condensed than in the previous stages.

During the diplotene stage, also known as diplonema from Greek words meaning "two threads", the homologous chromosomes separate from one another a little (Snustad & Simmons, 2008). The chromosomes themselves also uncoil a bit, allowing some transcription of DNA. However, the homologous chromosomes of each bivalent remain tightly bound at chiasmata, the regions where crossing-over occurred. The chiasmata remain on the chromosomes until they are severed in anaphase I.

In diakinesis, named from Greek words meaning "moving through", chromosomes condense further, and reaching maximum condensation (Snustad & Simmons, 2008). This is the first point in meiosis where the four chromatids of the tetrads are actually visible. Sites

of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Diakinesis closely resembles prometaphase of mitosis: the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form. Bivalents begin to migrate to the equator, guided by the spindle. When the bivalents reach the equator, the cell is said to be in metaphase I.

Thus, the chromosomes of any organism become increasingly condensed as they proceed through the substages of prophase I (Page & Hawley, 2003). Essentially, the chromosomes develop into "patches" in a "nucleoscape" before the metaphase I stage is reached. Could a method used to quantify the patchiness of chromosomal condensation provide a numerical predictor for metaphase I? Could the continuum of meiosis, or at least aspects of the continuum, be quantified?

### 1.3 Quantifying patchiness: Traditional methods versus the fractal dimension, $D_f$

The detection and quantification of spatial pattern, including the degree of patchiness of a landscape, is frequently undertaken in ecological studies. Methods to detect and measure special heterogeneity and patchiness calculate various multidimensional configurational indices that consider size, shape, patch density, and connectivity, to name a few variables (Gustafson, 1998). However, these typical "go-to" methods for detecting patchiness in landscape ecology are scale-dependent: simply stated, magnification of a patch results in the loss of the patchy pattern over the landscape, as one essentially "enters" the patch (or non-patch region), which then becomes the new prevalent, homogeneous environment (Kenkel & Walker, 1993). Likewise, what seem like large patches at one scale might appear like random bits of noise with a more distant perspective. This problem of scale is relevant to the idea of quantifying chromosomal patchiness: magnification with a microscope would also obscure patterns of patchiness. And what magnification is ideal for patch detection and measurement? Any measure of chromosomal patchiness must not depend on the scale.

Conveniently, the fractal dimension,  $D_f$ , is scale-independent (Julien & Botet, 1987). A fractal is an object or quantity that displays self-similarity on all scales. The object need not exhibit exactly the same structure at all scales, but the same "type" of structures must appear on all scales. The prototypical example for a fractal is the length of a coastline measured with different rulers of different lengths (Mandelbrot, 1967). A shorter ruler measures more of the sinuosity of bays and inlets than a larger one, so the estimated length continues to increase as the ruler length decreases. Plotting the length of the ruler versus the measured length of the coastline on a log-log plot gives a straight line, the slope of which is the fractal dimension of the coastline,  $D_f$ . In familiar Euclidean space, a line has 1 dimension, a plane has 2, and a cube, 3. However, the dimension of a fractal "line" in a two-dimensional surface has value of  $1 \leq D_f \leq 2$  (Julien & Botet, 1987; Kenkel & Walker, 1993). A  $D_f$  that approaches 1 implies that the particular object of interest (be it a landscape feature or chromosomal material) is found in patches and is less space-filling, whereas a  $D_f$  that approaches 2 suggests the measured feature is space-filling and dispersed, so that the overall landscape (or nucleoscape!) is not patchy (Sugihara & May, 1990).

The fractal dimension  $D_f$  has been used to quantify patchiness in landscapes, and often in digital landscape maps such as Landsat images (e.g., Lam, 1990; Milne, 1992), which, as will become evident, is relevant to the work described in this chapter. In analyzing digital landscape maps, a useful calculation is the "mass dimension", which describes the number

of grains or digital pixels of a given type occurring within a square window of size  $L \times L$  (Julien & Botet, 1987; Milne, 1992; Kenkel & Walker, 1993). A series of windows of size  $L \times L$  can be “slid” across the map, centring one on each pixel of the map that represents the object of interest. Within each window, the number of pixels of the object of interest ( $n$ ) is then counted. The procedure is repeated across a range of length scales. Because large windows will be limited by the extent of the map, only the data sampled by all window sizes can be included. These measures can then be used to quantify the statistical behaviour of the pattern. From the geometric measures described, a probability density function,  $P(n, L)$ , can be defined, which describes the probability of finding  $n$  pixels in a window of size (length)  $L$ . This probability function is analogous to a standard statistical distribution. The first moment of the probability distribution,  $M(L)$ , equivalent to mass dimension, is determined for increasing values of  $L$ ; the slope of the plot of  $\log M(L)$  vs.  $\log (L)$  represents  $D_f$ .

Could  $D_f$  be used to measure patchiness in a nucleoscape? A role for  $D_f$  in the quantification of meiosis seems possible: the  $D_f$  of nuclei in early prophase I, in leptotene, should approach 2, as the chromosomal material would be relatively dispersed. The  $D_f$  of nuclei progressing through zygotene, pachytene, diplotene, and diakinesis should decrease and approach 1 as the chromosomal material becomes more condensed, reaching maximal condensation at diakinesis. Furthermore, some value of  $D_f$  near 1 would likely be a quantitative indicator for the onset of metaphase I, especially as the alignment of chromosomes along the equator would be more organized, and essentially represent one giant cluster or “super-patch”. One of the authors of this chapter has done preliminary work toward this end (Ross 2005), and the results were promising enough to warrant the larger-scale study presented here.

#### 1.4 Megasporogenesis and microsporogenesis in three flowering plants as the systems for study

Megasporogenesis in flowering plants, the process by which a megasporocyte undergoes meiosis to produce spores -- typically of which only one goes on to produce the megagametophyte and egg -- provides a useful system for studying meiosis, particularly the prolonged and complicated prophase I. *Arceuthobium americanum* Nutt. ex Englm., the lodgepole pine dwarf mistletoe, is a particularly interesting organism and one that will provide a system in which to examine  $D_f$  as a measure of megasporocyte meiosis. The genus *Arceuthobium* comprises 42 species of economically-significant flowering plants that parasitize members of Pinaceae and Cupressaceae throughout North America, Central America, Asia, and Africa (Hawksworth *et al.*, 2002; Jerome & Ford, 2002). *Arceuthobium americanum* is found in North America, where it has the widest geographical range of any North American *Arceuthobium* species (Jerome & Ford, 2002), and can be found growing on two principal hosts, lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*Pinus banksiana*). The plant is dioecious, having female (Fig. 1A) and male flowers (Fig. 1B) on separate individuals (Hawksworth & Wiens, 1996), and a diploid number of 28 ( $2n = 28$ ) chromosomes (Wiens, 1964). Notably, prophase I in the megasporocyte *A. americanum* female flower is especially protracted: the megasporocyte enters prophase I (leptotene) at the end of one summer, goes into a resting period over the winter, and resumes prophase I (zygotene) in the next year's growing season before proceeding into metaphase I and the rest of meiosis (Hawksworth & Wiens, 1996; Ross & Sumner, 2004; Ross, 2005). As prophase I is so prolonged, the ability to predict metaphase I within days is a considerable challenge, and hence presents the perfect opportunity to test the ability of  $D_f$  to do so.



Two other species, similar to each other but decidedly different from *Arceuthobium americanum* should also be studied in order to capture more variability. The two species ideal for study are *Decaisnea insignis* (Fig. 1C) and *Sargentodoxa cuneata* (Fig. 1D), members of the Lardizabalaceae found in China and other Southeast Asian countries. Both are “winter bud” species that undergo megasporogenesis over three weeks (typically the last three in November) in the late fall (Wang *et al.*, 2009a, 2009b, & unpubl. data).

*Decaisnea* is a monotypic genus, with the species, *Decaisnea insignis* (Griffith) Hook. f. & Thomson, widely distributed from central to south-western China, extending into Bhutan, Myanmar, Nepal, Sikkim and north-eastern India (Chen & Tatemi, 2001). The plant is nicknamed ‘dead man's fingers’, as it possesses racemes of striking deep purplish-blue elongated fruits (follicles). The plant is economically important, as it is readily cultivated as an ornamental, and its fruits are deemed a delicacy. Plants are polygamo-monoecious, as individuals possess female flowers, male flowers and bisexual flowers (Wang *et al.*, 2009b). *Decaisnea insignis* has a diploid number of 30 ( $2n = 30$ ) chromosomes (Wu, 1995).

Like *Decaisnea*, *Sargentodoxa*, is also generally agreed to be a monotypic genus of the Lardizabalaceae (Rehder & Wilson, 1913; Chen & Tatemi, 2001; Soltis *et al.*, 2000; Angiosperm Phylogeny Group [APG], 2003), consisting of the single species, *Sargentodoxa cuneata* (Oliver) Rehder and E. H. Wilson. It has a wide distribution in China, and can also be found in Laos and Vietnam. The plant has ethnobotanical significance, having been used in folklore and medicine as a treatment for anemia and numerous human parasites, among other ailments (Huang *et al.*, 2004). Both male and bisexual flowers are found on the same individual, and thus the species is considered both dioecious and monoecious (Shi *et al.*, 1994). The functionally unisexual flowers are morphologically bisexual, at least developmentally, and the diploid number is 22 ( $2n = 22$ ) chromosomes (Wu, 1995).

Microsporogenesis, the process by which immature haploid pollen grains are formed by meiosis of the microsporocyte, is another useful process in which to study meiotic events, including the substages of prophase I. In fact, karyotypes for many plants are derived from studies of microsporocyte meiosis (*e.g.*, Wiens, 1964). Microsporocytes are technically much easier to harvest from flowers than megasporocytes, and as such,  $D_f$  can be determined for microsporocytes from several plant species undergoing prophase I and reaching metaphase I. *Arceuthobium americanum* also presents itself as a useful organism for study in this capacity, as microsporocytes in the male flower undergo meiosis rather rapidly over about three weeks at the end of the summer (Sereda, 2003). The resultant microspores (immature pollen) initiate microgametogenesis, but then overwinter prior to being released as mature pollen in the spring of the following year. The rapidity of meiosis in the microsporocytes should provide interesting fodder for comparison with the sluggish process in the *A. americanum* megasporocytes. Due to relative ease of obtaining microsporocytes, *Decaisnea insignis* (Fig. 1C) and *Sargentodoxa cuneata* (Fig. 1D) can also be mined for microsporocytes and  $D_f$  calculation. As with megasporogenesis, these species undergo microsporogenesis in the late fall, in the last three weeks in November (Wang *et al.*, 2009a, 2009b, & unpubl. data).

### 1.5 Aims and concerns

The objective of this work is to (1) calculate  $D_f$  for megasporocytes and microsporocytes from *Arceuthobium americanum*, *Decaisnea insignis*, and *Sargentodoxa cuneata* throughout

prophase I and upon the onset metaphase I; (2) to assess the ability of  $D_f$  to characterize the subphases of prophase I; (3) to evaluate the ability of  $D_f$  to predict the onset of metaphase I; and (4) to compare  $D_f$  between microsporogenesis and megasporogenesis amongst the three species, where informative. The ultimate goal will be to see if there is a universal value for  $D_f$  for either or both processes at some point, or across the species.



Fig. 1. Species used in this study. A) *Arceuthobium americanum* (female plant). B) *Arceuthobium americanum* (male plant). C) Portion of *Decaisnea insignis* shrub with inflorescences. D) *Sargentodoxa cuneata* inflorescence showing female flowers borne above the male. Scale bar = 15 mm in A, 5mm in B, 15 cm in C, and 9mm in D.

## 2. Materials and methods

### 2.1 Collection and preparation for microscopy

*Arceuthobium americanum* female flowers were collected daily from 1 March (prior to resumption of prophase I) until 1 May (when metaphase I has been reached or surpassed) in 2000 and 2001 from a stand of heavily infested jack pine in Belair, Manitoba, Canada as described in Ross (2005). Male *A. americanum* flowers were collected daily from August 14 until August 31 from infested lodgepole pine in Stake Lake, British Columbia, Kamloops.

Male and female flowers of *Decaisnea insignis* were collected daily from Taibai Mountain in Shaanxi Province, China 1 March 2005 to 1 May 2006 in order to obtain a range of developmental stages, although further work pinpointed the time of both megasporogenesis and microsporogenesis to about a three-week period at the end of November. Special attention was paid to when prophase I and metaphase I occurred. Only unisexual flowers were examined, even though the species sports bisexual flowers as well. Likewise, male and bisexual flower of *Sargentodoxa cuneata* were collected daily from the Nanchuan district of Chongqing city, China from 1 March 2006 to 1 May 2007, and as in *D. insignis*, further work pinpointed the time of both megasporogenesis and microsporogenesis to a three-week period at the end of November. Again, the occurrence of prophase I and metaphase I was noted. Only female components of the bisexual flowers were examined.

Flowers/buds for studies of megasporogenesis were fixed in formaldehyde/acetic acid/ethyl alcohol (FAA). Ovules were dissected from the ovaries, cleared in "4½" clearing solution (Herr, 1971) and mounted directly in Hoyer's medium on glass slides (Alexopoulos & Benke, 1952). Flowers/buds for the study of microsporogenesis were fixed in 1:3 acetic acid/ethanol, and the microsporocytes were spread directly on to glass slides with a dissecting needle. No staining was used, and the specimens were viewed with phase-contrast microscopy and consistent illumination. At least 5 megasporocytes and 5 microsporocytes were examined from each species per day.

## 2.2 Calculation of $D_f$

Whole megasporocytes or microsporocytes were digitized as binary (pure black and white) raster images with Adobe Photoshop® (resolution of 2400 dots per inch). Each image was cropped to include only the nucleus (nucleolus, if present, was ignored) and exported through NIH Image (Ross 2005) or Image J (Version 1.38) as a text file (black pixel=1, white pixel=0). This effectively converted chromosomes into black pixels, and any other nuclear material into white ones. Methods for the calculation of  $D_f$  were as described in Ross (2005).

## 2.3 Data management and statistical analyses

All statistical work was done with Minitab®. The mean value of  $D_f \pm$  standard error was calculated for the ~5 megasporocyte nucleoscares for each day, considering each species individually, and, in the case of *A. americanum*, separately for its two sampling years (first reported in Ross, 2005). Likewise, the mean value of  $D_f \pm$  standard error was calculated for the ~5 microsporocyte nucleoscares for each day, also individually for each of the three species. A two-tailed student's *t*-test (was used to compare the mean value of  $D_f$  from one day to the next, separately for each cell type (megasporocyte or microsporocyte) and species, to determine if the change in  $D_f$  was significant or not. A two-tailed student's *t*-test was also used to compare the values of *A. americanum* megasporocyte  $D_f$  between the two sampling years for days where the  $D_f$  values appeared similar but needed statistical verification.

To compare amongst all datasets, a one-way analysis of variance ANOVA was performed across the datasets for the start and end mean values of  $D_f$ . Then, to fairly assess how similar the  $D_f$  value was during the time period in which  $D_f$  was changing significantly across the species and cell types, that time period was standardized (day number/total days of significant change), and a one-way ANOVA was performed for values of  $D_f$  across all datasets for each standardized time.



### 3. Results

#### 3.1 $D_f$ for megasporogenesis and microsporogenesis in each species

As was described in Ross (2005) but reinterpreted here, megasporocyte nucleoscapes from *A. americanum* were examined daily from 1 March to 1 May in both 2000 and 2001; thus the examination period was 66 days for each year. The mean  $D_f$  for the 5 *A. americanum* megasporocyte nucleoscapes from the first date of sampling (1 March) was 1.903 ( $\pm 0.004$ ) in 2000 and 1.900 ( $\pm 0.003$ ) in 2001 (Fig. 2); these values are not significantly different ( $p > 0.95$ ). Visually, the chromosomal material appeared to be in leptotene and was relatively dispersed, (Fig. 3A), as would be anticipated for a mean  $D_f$  that approached 2. Mean  $D_f$  remained constant ( $\pm 0.003$ , 2000 and 2001 values equivalent,  $p > 95\%$ ) over the next 9 days in 2000 and over the next 13 days in 2001 (Fig. 2). However, mean  $D_f$  then made a significant drop ( $p < 5\%$ ) to 1.614 ( $\pm 0.002$ ) on 11 March 2000 and 1.606 ( $\pm 0.003$ ) on 15 March 2001 (Fig. 2); the “drop” values for 2000 and 2001 are equivalent ( $p > 95\%$ ), and the drop corresponded with the chromosomal condensation associated with the resumption of prophase I, likely zygotene (Fig. 3B). The mean  $D_f$  continued to decline significantly ( $p < 5\%$ ) each day over the next 38 days in 2000 (until 18 April) and over the next 32 days in 2001 (until 16 April), reaching statistically-similar ( $p > 99\%$ ) values of 1.352 ( $\pm 0.002$ ) in 2000 and 1.354 ( $\pm 0.002$ ) in 2001. At that time, the chromosomes were highly condensed but not yet at the equator, and were likely at diakinesis (Fig. 3B). The mean  $D_f$  continued to decline significantly ( $p < 5\%$ ) for the next 3 days until 21 April in 2000 (a total of 42 days of change) and for the next 3 days until 19 April in 2001 (total 36 days of change) to reach what would be a low of 1.332 ( $\pm 0.001$ ) in 2000 and 1.331 ( $\pm 0.001$ ) in 2001 (Fig. 2); values equivalent ( $p > 95\%$ ). At the end of this period of change, the chromosomes were at the equator and cells were in metaphase I. The average period of change between the two years was thus 39 days. After metaphase I was attained, the mean  $D_f$  did not change significantly ( $p > 99\%$ ) over the remainder of the examination period. Therefore, a  $D_f$  (both years considered) of about 1.353 ( $\pm 0.003$ ) corresponded with diakinesis, which predicted metaphase I in *A. americanum* megasporocytes by 3 days in both study years, and  $D_f$  at metaphase I was the lowest stable value of about 1.332 ( $\pm 0.002$ ), both years considered.

Mean  $D_f$  for 5 megasporocyte nucleoscapes from *D. insignis* and *S. cuneata* (Fig. 4) as well as 5 microsporocyte nucleoscapes from these two species (Fig. 5) were determined each day from a period 13 November to 30 November (2005 for *D. insignis*, 2006 for *S. cuneata*); a total examination period of 18 days for each species. Similarly, mean  $D_f$  for 5 *A. americanum* microsporocyte nucleoscapes were determined each day from 14 August to 31 August 2010 (Fig. 6), also 18 days of study. The results were startlingly uniform across the abovementioned species and the cell types. The mean  $D_f$  at the beginning of the examination period ranged between 1.902 and 1.909 ( $\pm 0.008$ ) for these species and cells, and reached a low of 1.330 to 1.332 ( $\pm 0.003$ ) when the cells were in metaphase I (Figs. 4 to 6). The period in which mean  $D_f$  was changing was 9 days. In all cases, when the mean  $D_f$  had reached 1.355 to 1.360 ( $\pm 0.006$ ), the cells were apparently in diakinesis, and metaphase I occurred the day after diakinesis was reached. Notably, the high values, diakinesis values, and low values were all statistically similar to each other, respectively, and also to the values for *A. americanum* megasporocytes (Fig. 2) described previously ( $p > 90\%$ ).

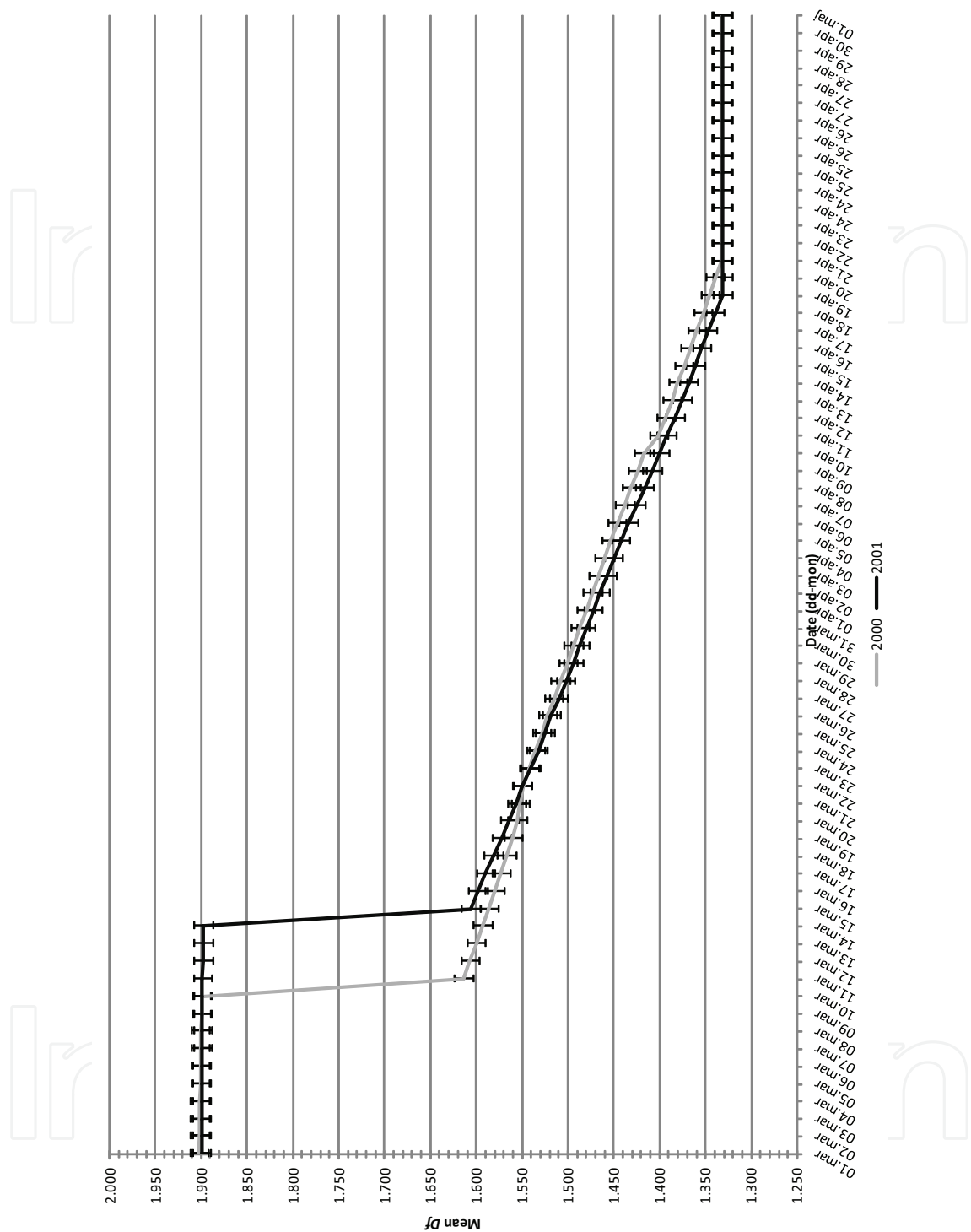


Fig. 2. Daily value of mean  $D_f$  from 1 March to 1 May in 2000 and 2001 for *Arceuthobium americanum* megasporocytes undergoing prophase I. Mean  $D_f$  makes a significant drop ( $p<5\%$ ) to 1.614 ( $\pm 0.002$ ) on 11 March (2000) and 1.606 ( $\pm 0.003$ ) on 15 March (2001), continues to decline significantly ( $p<5\%$ ) each day over the next 41 days in 2000 (until 21 April) and next 35 days in 2001 (until 19 April), to reach a stable low value of 1.332 ( $\pm 0.001$ ) in 2000 and 1.331 ( $\pm 0.001$ ) in 2001. There were thus 39 average total days of change.

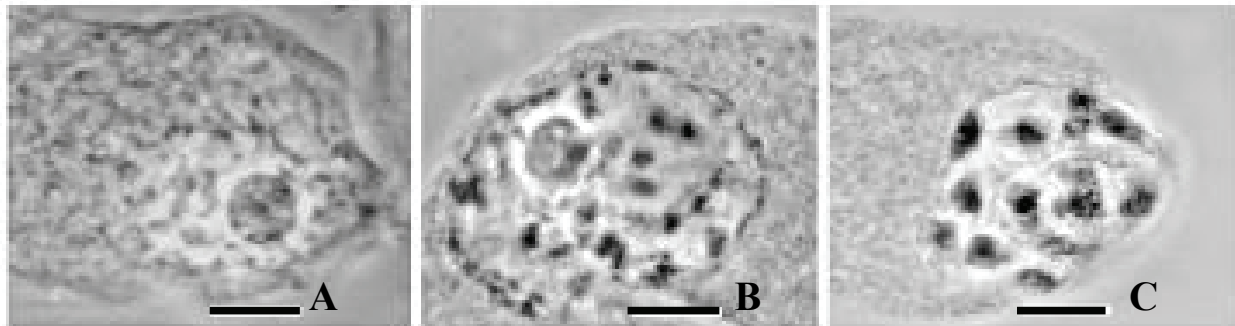


Fig. 3. Phase-contrast light micrographs of meiotic nuclei in megasporocytes of the dwarf mistletoe, *Arceuthobium americanum*. A) Nucleus sampled prior to the resumption of prophase I (near leptotene). B) Nucleus sampled when prophase I resumes (zygotene), chromosomal condensation commences, and the value of the fractal dimension,  $D_f$ , begins to drop. C) Chromosomes are maximally condensed, cell is in diakinesis. Metaphase I will occur in 3 days, and  $D_f$  will reach its lowest value. Scale bar = 10  $\mu$ m in A, B, C. (Used with permission from Ross (2005) and the *International Journal of Biological Sciences* ISSN 1449-2288).

Specifically, mean  $D_f$  in both *D. insignis* and *S. cuneata* megasporocytes made a significant drop ( $p < 5\%$ ) to  $1.592 (\pm 0.002)$  on 19 November (Day 7) and to  $1.594 (\pm 0.001)$  on 16 November (Day 4), respectively, and then continued to decrease significantly over the next 7 days, at which time diakinesis was achieved, with the mean  $D_f$  becoming  $1.357 (\pm 0.002)$  on 26 November (Day 14) in *D. insignis* and  $1.355 (\pm 0.002)$  on 23 November (Day 11) in *S. cuneata* (Fig. 4). Chromosomal condensation from leptotene (Fig. 7A) to diakinesis (Fig. 7B) was accordingly evident in the megasporocytes (Fig. 7 shows an example from *D. insignis*). The next day, 27 November (Day 15) in *D. insignis* and 24 November (Day 12) in *S. cuneata*, the lowest mean  $D_f$  was reached in both species ( $1.331 \pm 0.002$  for *D. insignis* and  $1.330 \pm 0.001$  for *S. cuneata*), and the megasporocytes had reached metaphase I (Fig. 4). Thus, the period of change for mean  $D_f$  was, as mentioned 9 days (inclusive).

Similarly, mean  $D_f$  in *A. americanum*, *D. insignis*, and *S. cuneata* microsporocytes made a significant drop ( $p < 5\%$ ) to  $1.595 (\pm 0.003)$  on 18 August (Day 5), to  $1.597 (\pm 0.002)$  on 17 November (also Day 5), and to  $1.595 (\pm 0.001)$  on 18 November (Day 6), respectively, and then continued to decrease significantly for the next 7 days, reaching diakinesis on 25 August (Day 12), 24 November (also Day 12), and 25 November (Day 13) with mean  $D_f$  values of  $1.358 (\pm 0.003)$ ,  $1.360 (\pm 0.002)$ , and  $1.357 (\pm 0.003)$ , respectively (Figs. 5 and 6). As in the megasporocytes, according chromosomal condensation from leptotene (Fig. 8A) to diakinesis (Fig. 8B) was evident in the microsporocytes (Fig. 8 shows an example from *S. cuneata*). The day after diakinesis occurred, microsporocytes in *A. americanum*, *D. insignis*, and *S. cuneata* reached metaphase I, specifically 26 August (Day 13), 25 November (also Day 13) and 26 November (Day 14), respectively, with respective lowest mean  $D_f$  values (Figs. 5 and 6) of  $1.330 (\pm 0.001)$ ,  $1.329 (\pm 0.002)$ , and  $1.332 (\pm 0.002)$ . Again, as was the case for *D. insignis* and *S. cuneata* megasporocytes,  $D_f$  decreased over a 9-day period in the microsporocytes for all three species examined.

### 3.2 Comparing $D_f$ over a standard time frame: Have we some universal values?

The time frame for the progression of prophase I into metaphase I in *Arceuthobium americanum* megasporocytes was much longer than in its microsporocytes and longer than

the period in both the megasporocytes and microsporocytes of *Decaisnea insignis* and *Sargentodoxa cuneata*. Specifically, the period for which mean  $D_f$  experiences significant daily drops in *A. americanum* megasporocytes averaged 39 days, but was only 9 days for the others. However, it is patently clear that  $D_f$  has statistically-significant similar value and meaning for meiosis across the cells and species studied here. Furthermore, when the time frame of mean  $D_f$  is standardized for each cell type and species, the values are all statistically equivalent ( $p>95\%$ ). This similarity is also evident in Fig. 9, which depicts the change in  $D_f$  for both cells and all species studied. Most notably, the slope of the regression line for the averaged values is -0.289, and the Y intercept for the regression line (the value at which mean  $D_f$  begins to drop) is 1.602. It is this slope which could likely be used to predict substages of prophase I and metaphase I. Other key predictive values of  $D_f$  are given in Table 1.

Mean $D_f \pm$ Standard Error	Event
1.904 $\pm$ 0.009	Prophase I (leptotene)
1.599 $\pm$ 0.008	Prophase I (zygotene)
1.356 $\pm$ 0.003	Prophase I (diakinesis)
1.331 $\pm$ 0.004	Metaphase I

Table 1. Mean  $D_f$  averaged across megasporocytes and microsporocytes in *Arceuthobium americanum*, *Decaisnea insignis*, and *Sargentodoxa cuneata* as predictors (all were statistically similar,  $p>95\%$ ).

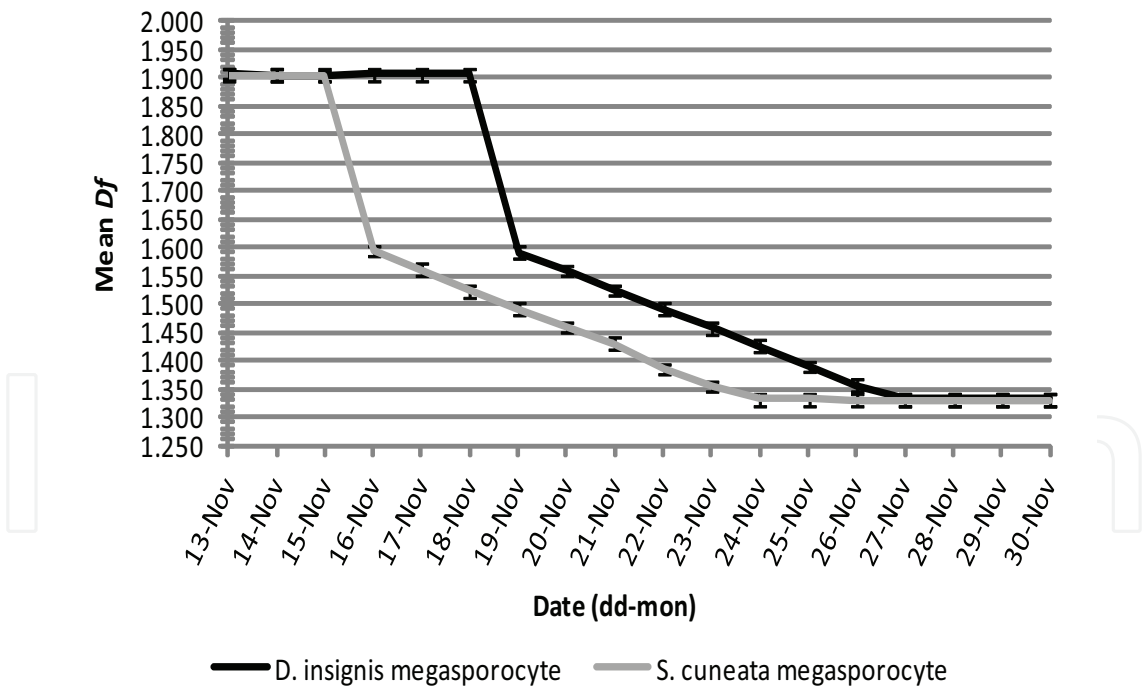


Fig. 4. Daily value of mean  $D_f$  from 13 November to 30 November for *Decaisnea insignis* (2005) and *Sargentodoxa cuneata* (2006) megasporocytes undergoing prophase I. Mean  $D_f$  makes a significant drop ( $p<5\%$ ) to 1.592 ( $\pm$ 0.002) on 19 November (*D. insignis*) and to 1.594 ( $\pm$ 0.001) on 16 November (*S. cuneata*), continues to decline significantly ( $p<5\%$ ) each day over the next 8 days in both species (until 27 November and 24 November, respectively), to reach a stable low value of 1.331 ( $\pm$ 0.002) and 1.330 ( $\pm$ 0.001), respectively (total 9 days of change).



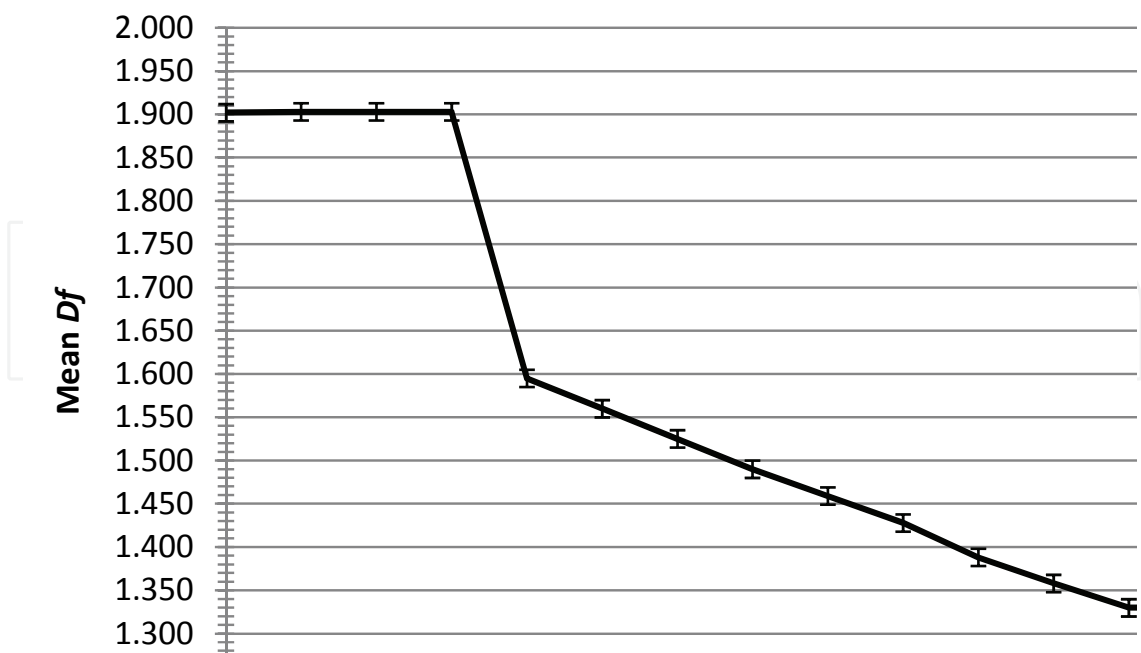


Fig. 5. Daily value of mean  $D_f$  from 14 August to 31 August in 2010 for *Arceuthobium americanum* microsporocytes undergoing prophase I. Mean  $D_f$  makes a significant drop ( $p<5\%$ ) to 1.595 ( $\pm 0.003$ ) on 18 August, continues to decline significantly ( $p<5\%$ ) each day over the next 9 days (until 26 August), to reach a stable low value of 1.330 ( $\pm 0.001$ ), 9 days of changing  $D_f$ .

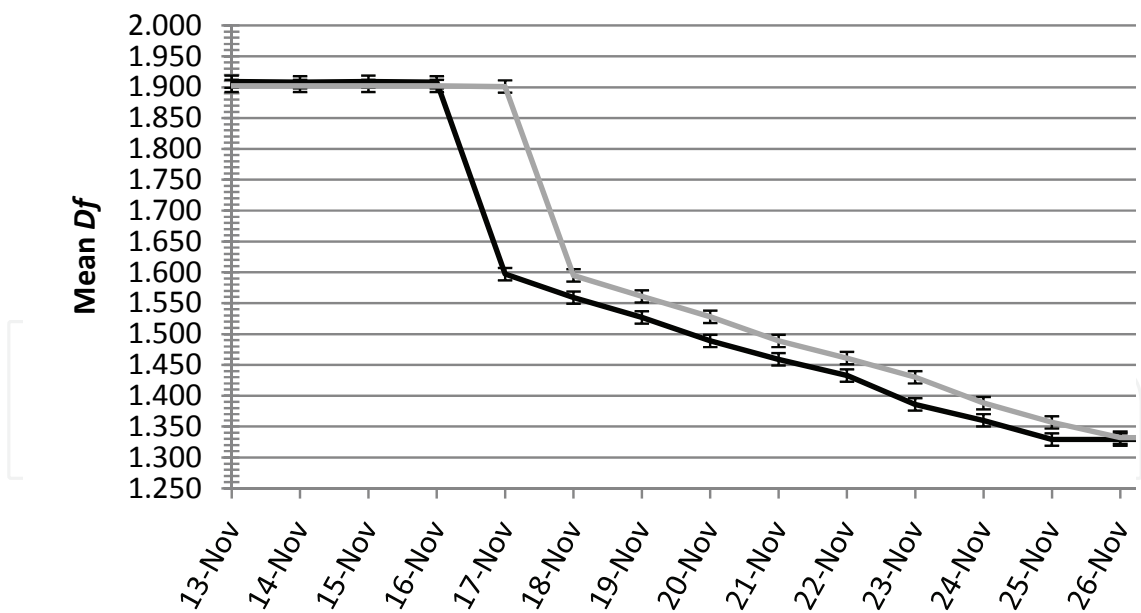


Fig. 6. Daily value of mean  $D_f$  from 13 November to 30 November for *Decaisnea insignis* (2005) and *Sargentodoxa cuneata* (2006) microsporocytes undergoing prophase I. Mean  $D_f$  makes a significant drop ( $p<5\%$ ) to 1.597 ( $\pm 0.002$ ) on 17 November (*D. insignis*) and to 1.595 ( $\pm 0.001$ ) on 18 November (*S. cuneata*), continues to decline significantly ( $p<5\%$ ) each day over the next 8 days in both species (until 25 November and 26 November, respectively), to reach a stable low value of 1.329 ( $\pm 0.002$ ) and 1.332 ( $\pm 0.002$ ), respectively (total 9 days of change).

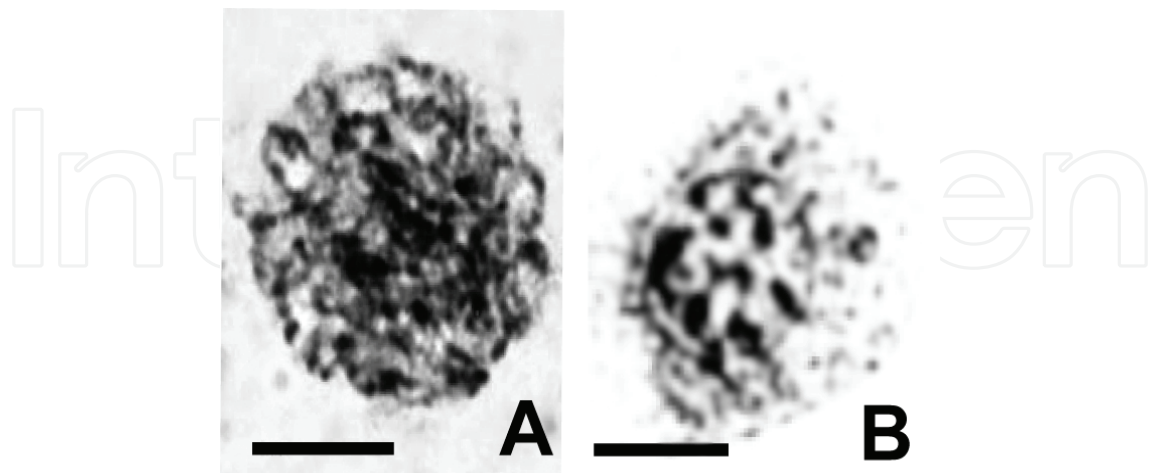


Fig. 7. Phase-contrast light micrographs of meiotic nuclei in the megasporocytes *Decaisnea insignis*. A) Nucleus sampled prior to the resumption of prophase I (near leptotene). B) Nucleus sampled when prophase I resumes (zygotene), chromosomal condensation commences, and the value of the fractal dimension,  $D_f$ , begins to drop. Metaphase I will occur in 1 day, and  $D_f$  will reach its lowest value. Scale bar = 15  $\mu\text{m}$  in A, 30  $\mu\text{m}$  in B.

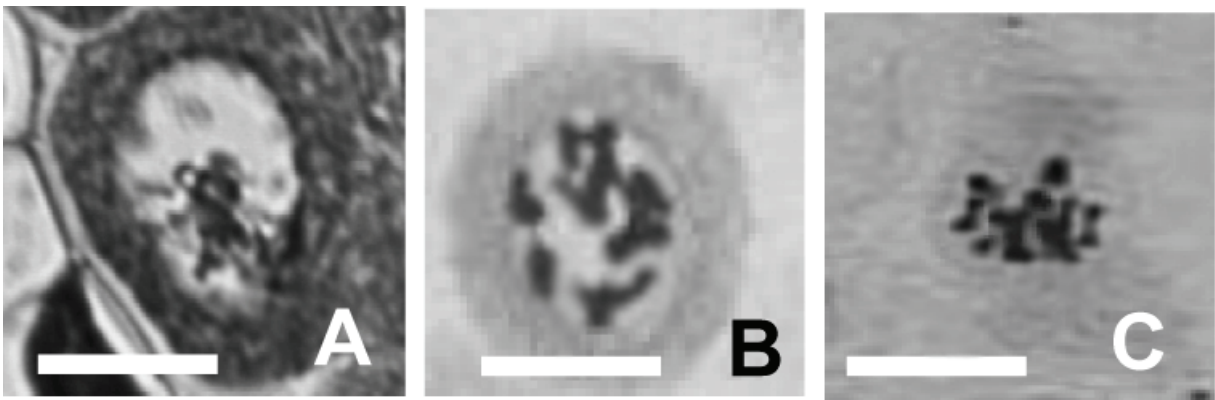


Fig. 8. Phase-contrast light micrographs of meiotic nuclei in the microsporocytes of *Sargentodoxa cuneata*. A) Nucleus sampled prior to the resumption of prophase I (near leptotene). B) Chromosomes are maximally condensed, cell is in diakinesis, and the value of the fractal dimension,  $D_f$ , has reached a low value that predicts metaphase I by one day. C) Nucleus is in metaphase I, one day after diakinesis was reached, and  $D_f$  has reached its stable, lowest value. While chromosomes are maximally condensed in B, their position along the equator drove  $D_f$  to its lowest value. Scale bar = 30  $\mu\text{m}$  in A, B, C.

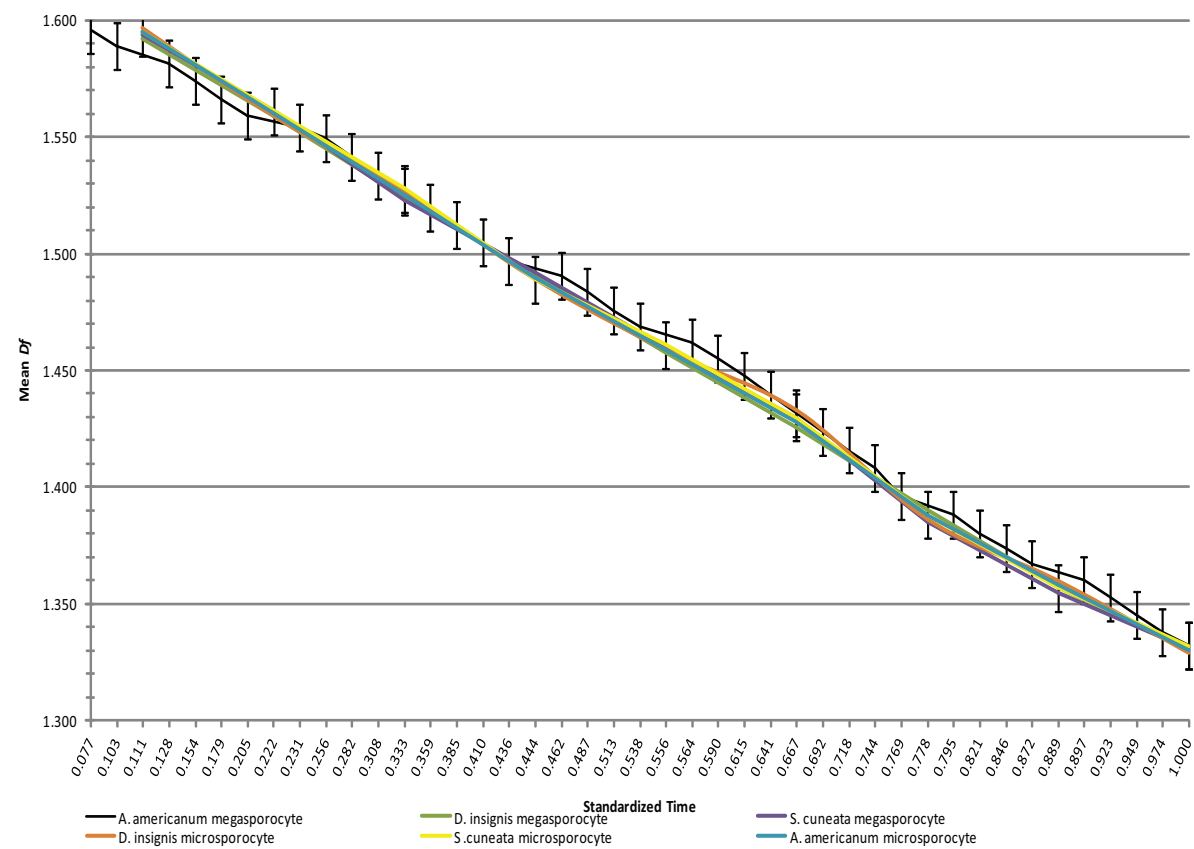


Fig. 9. Daily value of mean  $D_f$  over the standardized period of change (day number/total days of significant change) for *Arceuthobium americanum*, *Decaisnea insignis*, and *Sargentodoxa cuneata* megasporocytes and microsporocytes undergoing prophase I. Values for *A. americanum* megasporocytes were averaged over the two years of study (2000 and 2001). The values follow a statistically-similar linear trendline. Slope of the regression line = -0.289; Y intercept = value at which mean  $D_f$  begins to drop = 1.602 for the regression line.4.

4. Discussion

In the work done here with *Arceuthobium americanum*, *Decaisnea insignis*, and *Sargentodoxa cuneata* megasporocytes and microsporocytes undergoing meiosis, the fractal dimension  $D_f$  described the relative degree of chromosomal condensation in prophase I to metaphase I more sensitively and quantitatively than microscopic observation alone. Across all species and cell types, a  $D_f$  of  $1.904 \pm 0.009$  described leptotene of prophase I,  $D_f$  of  $1.599 \pm 0.008$  delimited zygotene of prophase I, and  $D_f$  of  $1.356 \pm 0.003$  characterized diakinesis. A  $D_f$  approaching 1 is consistent with a nucleoscape made patchy with highly condensed chromosomal material, as would be the case for patches in a landscape (Sugihara & May, 1990). Even though the timing was different with respect to the *A. americanum* megasporocytes, the values across all species and both cell types were statistically similar. Interestingly, the timing of prophase I and the onset of metaphase I in microsporocytes across the three species as well as the timing of the same stages in the megasporocytes in *D. insignis* and *S. cuneata* was astonishingly similar: prophase seemed to occur in 9 days in all of these. One might have thought that the disparities amongst the species, particularly

regarding microsporogenesis between *A. americanum* and the two members of Lardizabalaceae, would have resulted in different timing. However, it might be that these three species have similar amounts of DNA, as nuclear DNA content is known to be important factors determining or affecting the duration of male meiosis (Bennett *et al.*, 1973). These workers found that the timing of microsporogenesis and, to a certain extent, megasporogenesis, was similar in *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), and *Secale cereale* L. (rye).

A mean  $D_f$   $1.331 \pm 0.004$  quantified metaphase I, which was also predicted by the  $D_f$  measured for diakinesis. In *A. americanum* megasporocytes, metaphase I occurred 3 days after reaching diakinesis, and in the remaining cells and species examined, it occurred one day after diakinesis. "Fractally speaking", a low value of  $1.331 \pm 0.004$  make sense as a descriptor of metaphase I: while the chromosomes are as about as condensed as they are in diakinesis, their clustering at the equator contributes to the patchiness, which in turn is captured in the calculation of  $D_f$  by the probability-density function. Ross (2005) described slightly different results for the same data, but had erroneously identified metaphase I as diakinesis. In other species not studied here, the timing might be different, but perhaps the the values of  $D_f$  calculated for these stages (Table 1) can be predictive.

One might argue that the mere occurrence of diakinesis could just as effectively predict metaphase I without the calculation of  $D_f$ , but it should be stressed that the rate of change of mean  $D_f$  as determined by the slope and determined within a few days during the decrease, one would be able to predict diakinesis as well as metaphase I! In other words, once  $D_f$  begins to drop (i.e., the Y-intercept value), one could easily calculate the slope of the line (which this study showed to be consistent across all species and both cell types at around -0.289) with a few days' worth of data, then estimate when the consistent metaphase I low of  $1.331 \pm 0.004$  will be reached. This is extremely powerful.

This study has shown that meiotic timing and events amongst different species can be compared quantitative fashion that will eliminate the subjectivity inherent in qualitative descriptions. The method for harvesting nuclei and extracting  $D_f$  is relatively simple, much more so than embedding and staining tissues, so time may be saved if many metaphase I slides are required. As prophase I is always prolonged relative to metaphase I, regardless of the organism, some value of  $D_f$  should be predictive in any species examined. The method as described here will work particularly well in other species with relatively long meiotic divisions, such as peonies, genus *Paeonia* (Shamrov, 1997). In commercially important plants such as these, predicting when meiotic events occur can be particularly important and could streamline the culture of zygotic embryos. The examination and calculation of  $D_f$  in other species and other stages of meiosis should be tackled.

Use of this technique should also allow for the quantification of *mitosis* (the equational nuclear division) along with the prediction of mitotic metaphase. As for meiosis, quantification of mitosis will facilitate objective comparisons of mitotic events amongst cells, tissues, individuals, and species. Notably, a quantification of mitosis could prove considerably valuable for another reason; if  $D_f$  values can be determined for normal tissues, it is likely that deviations in  $D_f$ , either in absolute value or rate of change, could signal abnormalities in the cell cycle that might be correlative to precancer events in animals.



Fractal analysis and chaos theory have been used to describe the histological texture of tumours (Mattfeldt, 1997), and so the work described in our study seems like it would be applicable to systems beyond the study of meiosis.

## 5. Conclusion

Key values of the fractal dimension ( $D_f$ ) is *Arceuthobium americanum*, *Decaisnea insignis*, and *Sargentodoxa cuneata* megasporocytes and microsporocytes undergoing meiosis were able to describe leptotene, zygotene, diakinesis, and metaphase I. When  $D_f$  began to drop, the rate of change across all species and both cell types was consistent (slope = - 0.289), and this slope could be used as a predictor for the abovementioned stages. The results are novel and exciting, and we hope they will be re-examined and extrapolated to other cells, species, processes, and systems.

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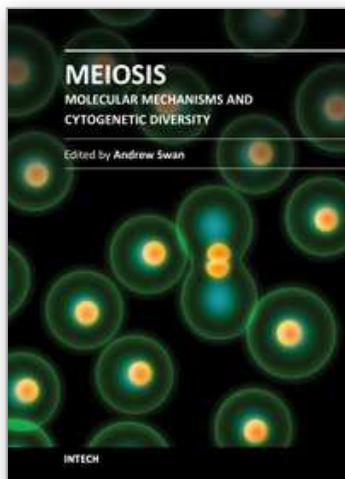
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Meiosis, the process of forming gametes in preparation for sexual reproduction, has long been a focus of intense study. Meiosis has been studied at the cytological, genetic, molecular and cellular levels. Studies in model systems have revealed common underlying mechanisms while in parallel, studies in diverse organisms have revealed the incredible variation in meiotic mechanisms. This book brings together many of the diverse strands of investigation into this fascinating and challenging field of biology.

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