

# **A Study on the Effect of Time in Plants: Within-Group Variation in Morphological Integration in Clover (Trifolium Repens L.) in Different Aged Pastures**

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## **ABSTRACT**

We are engaged in a continuing study concerning the effect of time on plants. The approach is to apply a common statistic, within-group variation in morphological integration, to plants of different age or subjected to different treatments in a search for common underlying patterns. Within-group variation in morphological integration is an estimate of the relationship among variables. Previous studies have shown that it increases with time, both in ontogeny and phylogeny, and also it is higher in grape leaves that develop in a higher energy environment. The clovers studied here add a period of time longer than ontogeny and shorter than phylogeny and thus expand the comparison of different aged plants. As with the effect of ontogeny or phylogeny, clovers collected from the oldest pasture (45 years old) had the highest within-group variation in morphological integration. This time-related change in within-group variation in integration is similar to that seen in grape leaves that grew in higher energy environments over a single growing season. Thus, it is tempting to consider these similar responses to time and energy as an argument for thermodynamics being the underlying cause of time-related changes in plants.

## 1. Introduction

Irreversible time-related phenomena are central to biological events. They are seen in the appearance of an individual, in ontogeny, or in an evolutionary lineage, phylogeny. It is the nature of time-related events that has formed the focus of research for one of us (Maze) for ten years, in particular the common features of ontogeny and phylogeny (see Maze *et al.* 2003a). This study expands our comparisons of time-related phenomena by analyzing time periods greater than those seen in ontogeny but shorter than those of phylogeny.

We chose to evaluate the effect of time by comparing the level of within-group variation in morphological integration. Within-group variation in morphological integration is a measure of the diversity among the growth curves from which a plant arises; as the variation in morphological integration increases the number of different expressions of those growth curves also increases (see Maze *et al.* 2003b). Within-group variation in integration is also an estimate of the variation in among-variable relationships. We use the degree of modification of growth curves that accompanies development or evolution as a way to approach time-related changes of form in plants. Within-group variation in morphological integration is also related to the concept of emergence (see Maze and Bohm 1997).

We have obtained consistent results from studies of time-related within-group variation in morphological integration. In studies using related grasses the within-group variation in morphological integration tends to increase with the level in a taxonomic hierarchy from single species to groups of related species (Maze 1998; Maze *et al.* 2001a; 2002a); higher levels in a taxonomic hierarchy are inferred to be older. Likewise, older leaf primordia in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) show higher within-group variation in morphological integration than do younger ones (Maze *et al.* 2000), as do older grass ovules in *Achnatherum nelsonii* (Scribn.) Barkworth and *A. letermanii* (Vasey) Barkworth (Maze *et al.* 2001b)

A relationship between higher amounts of within-group variation in morphological integration and the amount of energy processed has also been reported. For example, faster growing seedlings of Engelmann spruce (*Picea engelmannii* Parry), which were assumed to be processing more energy, showed a higher level of within-group variation in morphological integration (Maze *et al.* 2002b). Likewise, a higher within-group variation in morphological integration was demonstrated for grape leaves (*Vitis vinifera* L. var.

Merlot) that developed in a higher temperature regime than those that developed in a lower temperature regime (Maze *et al.* 2003b).

The intriguing principle inferred from these results is the similar response elicited by time (level in a taxonomic hierarchy, age of leaf primordia or grass ovules) and amount of energy processed (spruce seedlings, grape leaves). It is tempting to use these similar responses to energy and time to hypothesize a common feature underlying energy processing and the passage of time in biological material. To explore this potential common feature we wish to expand our comparisons to include more measures of time. It is the purpose of the present study to compare within-group variation in morphological integration in plants from younger and older pastures. The prediction is that plants from the older pasture will show a higher degree of within-group variation in morphological integration.

## 2. Materials and methods

### 2.1. Plants analyzed

Individual clovers were collected from three pastures near Aldergrove, ca. 55 km ESE of Vancouver, British Columbia, Canada (49° 03' 45" N, 122° 30' 45" W). The pastures were originally sown with a seed mixture of 5 - 10% *Trifolium repens* L., 15 - 20% *Dactylis glomerata* L. and 70 - 80% of "High-land" forage mixture from the Richardson Seed Co., Ltd. in Burnaby, British Columbia. The pastures were sown in 1939, 1958 and 1977. The collections were made in 1984 and provides us with clovers growing in pastures of known age, 7, 26 and 45 years, and known source. There will, of course, be some natural reseeding but for white clover this is negligible (Parish and Turkington 1989). Since their establishment, the three pastures have never received artificial fertilizer or herbicide, have had an annual application of cow manure, have been grazed by cattle (20 - 30 head from May until November) and occasionally cut for hay. The pastures planted in 1939 and 1977 are generally flat, although there is a slight slope in that planted in 1977, while there is a gentle rolling relief in the pasture planted in 1958; there was little among-pasture variation in edaphic factors (Aarssen and Turkington 1985).

Within each pasture 60 systematically placed 50 cm<sup>2</sup> quadrats were laid out. From each of the 180 quadrats, all *T. repens* were carefully removed and, as far as possible, each genet (individual plant) was removed intact. All intact genets were then cut into individual ramets. Each ramet was then propagated vegetatively in individual pots, 10 cm

diameter and 13 cm deep, that were randomly arranged in a plastic greenhouse at the South Campus Experimental Plots at the University of British Columbia. The plants were grown for about eight weeks, until they were well established. Once they had become well established then measurements were taken from each ramet. The data analyzed were the average of all the ramets for each genet (plant). There were 239, 312 and 262 plants analyzed for the 7, 26 and 45 year old pastures respectively.

## **2.2. Variables measured**

Seven variables were measured and used in the analyses: leaf width at the widest point across the lateral leaflets, petiole length from the same leaf, length of the internode proximal to the leaf measured, peduncle length, dry mass of the inflorescence from which peduncle length was taken, number of flowers from that same inflorescence and dry weight of twenty of its seeds. Five measurements were made for each ramet after the various parts chosen had ceased to expand. Because they were still elongating the three newest internodes were always ignored when making measurements.

## **3. Analyses**

Integration is an important biological phenomenon since (i) it is related to organization, that which gives organisms their unique features, and (ii) it allows functioning, the processing of matter and energy; for these reasons it is a valuable feature to offer insight into biological phenomena. To assess within-group variation in morphological integration we calculated the value ANG using Pimentel's (1993) MPCA program for the clovers from each of the three pastures. ANG, as used here is the angle formed between first eigenvectors for principal components analysis (PCA) for subgroups of clovers from any one pasture, and the first eigenvector from the pooled sample from that same pasture. The PCA used was of a variance/covariance matrix of log transformed variables ( $\ln[X+1]$ ) (Pimentel 1993). First eigenvectors from such a PCA are allometric coefficients (Jolicoeur and Mosiman 1960) so we are comparing relative growth rates for the variables measured within the clovers in any one pasture. A pasture in which the clovers have more divergent growth rates among the parts measured will produce greater values for ANG than a pasture in which the clovers show lower divergence in growth rates (see Maze *et al.* 2003a,b). This use of ANG is similar to what was called variation in organization in previous studies (e.g., Maze *et al.* 1998, 1999; Maze and Bohm 1997; Maze *et al.* 2000). Although the terms used are somewhat different the concepts addressed, within-group variation in morphological integration, remain the same.

To calculate ANG for each pasture, all clovers from that pasture were randomly divided into subgroups each of which would have an angle between its first eigenvector and the first eigenvector for all clovers from that pasture. For example, for the youngest pasture within which 8 subgroups were recognized, there would be 8 values for ANG and the within-group variation in morphological integration for the clovers in that pasture would be represented in those 8 values for ANG. By so allotting the clovers from each pasture we avoided pseudoreplication in a mensurative experiment by assuring that the samples are not "... more restricted than the inference space in the hypothesis being tested" (Hurlbert 1984, p. 190).

The middle aged and oldest pasture were each divided into 10 subgroups giving 10 estimates of within-group variation in morphological integration for each of those pastures (the number of subgroups was chosen to assure stability of PCAs which require a minimum sample size of 26 [Pimentel 1993]). The relationship between age and within-group variation in morphological integration (ANG) was evaluated using regression analysis. The important thing to remember about ANG is, not whether the angles for the subgroups are different from the pooled sample, but that the angles formed between subgroups and the pooled samples in a population subjected to different treatments, in this case pasture age, are different from each other. Or, ANG is a derived descriptive variable for a group grown under particular circumstances.

We also made a comparison of variable size in the three pastures. This was done because Maze *et al.* (2003b) demonstrated that those grape leaves that showed the highest degree of within-group variation in morphological integration were smaller. This was seen in leaves that developed in a higher energy environment and was attributed to the dissipation of energy through the establishment of more diverse developmental vectors. It was reasoned in Maze *et al.* (2003b) that if the added energy was not dissipated into larger leaves then it was dissipated into more diverse growth vectors. This is a phenomenon which will produce more diverse allometric coefficients in a sample. In the current study size was evaluated for each variable separately, whereas in Maze *et al.* (2003b) size was estimated by a summary variable, PCA axis scores. Individual variables were analyzed for size differences here since the first PCA axis from the clover data was a shape axis and not a size axis (Pimentel 1979).

If the results reported here are consistent with those from the grape leaves, we take it to indicate that the passage of time produces results that mimic the dissipation of energy.

This is intriguing because of a proposed relationship between thermodynamic phenomena and evolutionary change (Brooks and Wiley 1988).

The principal components analyses were done using Pimentel's (1993) MPCA program. The relationship between age and variable size and between within-group variation in morphological integration and age was estimated by regression analysis using SYSTAT (Wilkinson 1988).

## 4. Results

The results of the regression analyses of variables against age of the pastures reveal three patterns (Table 1).

- (i) a decline in variable value with age in leaf width, petiole length, internode length, peduncle length and number of flowers
- (ii) an increase in variable value with age in dry weight of seeds
- (iii) no change with age in dry mass of inflorescence.

**Table 1: Regression analysis of each variable on age of pasture. r<sup>2</sup>, coefficient of determination; slope, slope of regression line; p, probability slope=0.0; n=804**

	r <sup>2</sup>	slope	p
leaf width	0.045	-0.064	0.000
petiole length	0.046	-0.187	0.000
internode length	0.104	-0.087	0.000
peduncle length	0.211	-0.923	0.000
dry weight of inflorescence	0.003	0.000	0.114
number of flowers	0.086	-0.209	0.000
dry weight of seeds	0.143	0.035	0.000

Within-group variation in morphological integration increases significantly with age with a slope of 0.771, and with a coefficient of determination of 0.493 ( $p < 0.001$ ;  $n = 28$ ).

## 5. Discussion

Two patterns were detected in this study related to the increasing age of the pasture from which the clovers were collected. One pattern was a decrease in size in the measured variables, while the other was an increase in within-group variation in morphological integration. With increasing age, there would be a greater amount of energy, accumulated over time, which would have to be dissipated. Three ways exist to dissipate energy; increased production of heat, an increase in size as more energy is dissipated into matter or

an increase in the number of pathways through which energy may be dissipated. The first is not available to plants. The second would occur via an increase in the rate at which energy is converted to matter along existing growth vectors. The third would be the result of adding growth vectors. It is the third option that is seen in the clovers studied here, as energy is shunted away from existing growth vectors resulting in smaller individual parts. These observations are also congruent with those made previously on grape leaves developing under a higher energy regime (as measured in mean temperatures). The greater amount of energy was dissipated via the formation of new vectors of growth rather than an expansion in existing growth vectors (Maze *et al.* 2003b). That is, the increase in energy produced developmental novelty in grape leaves. Within-group variation in morphological integration reflects variation in relative growth rates among the variables which describe the plants being studied. This variation is captured in ANG, the statistic used to evaluate within-group variation in morphological integration.

Even though there is an increase in within-group variation in integration in response to the processing of more energy, the new variants produced are constrained by the existing boundary conditions, *sensu* Polanyi (1976) (the perimeters of their normative modes), within which energy dynamics are carried out. The different forms of clovers, and the grape leaves (see above), are subtle indeed, resembling the magnitude of plants growing at different elevations on a mountain side; i.e., the different groups are detectable only after detailed measurements and analyses designed to reveal subtle, and often complex, differences. The plants retain their basic morphology even though variants are produced in response to altered energy regimes, i.e., clovers remain clovers and grape leaves grape leaves. Should an altered energy environment “demand” a response beyond that of which a plant is capable, death would result, either of a part or of the whole plant.

The results recorded in these studies are similar responses by different plants to rather different events of biological significance; energy dynamics in grape leaves and time in clovers. In both cases the assessment of those biological events are indirect, i.e., energy dynamics being evaluated by different mean temperatures under which the leaves developed, and time by the age of a pasture in which the plants grew. Still the methods remain justifiable (Maze *et al.* 2003b). Using pasture age as an estimator of age in clovers growing there is acceptable given that the rate of reseeded of clover is negligible (Parish and Turkington 1989) and clover is a long-lived perennial plant.

The similar results encountered in these studies may be used to argue for some relationship between time-related changes in plants and those associated with thermodynamic

phenomena. Possibly this similarity between thermodynamic phenomena and time-related changes argues for a common underlying cause, as explored by Brooks and Wiley (1988) in arguing that evolution is a thermodynamic phenomenon. Energy dynamics in plants take place via matter transformation as expressed, e.g., in the fixation of light energy during photosynthesis and the formation of cell walls during ontogeny. The information emphasized by Brooks and Wiley (1988), is linked to energy dynamics via matter transformations of such types.

DNA is not independent of energy dynamics and may be viewed as a repository for the constraints on expression of some aspects of matter transformation, particularly those which accompany changes in ontogeny or reproduction. It is one of the boundary conditions, *sensu* Polanyi (1976), that determine how the inanimate laws of nature are expressed in plants. If species are a virtual code (see Maze *et al.* 2003c; 2005) then both the matter transformation accompanying energy dynamics and DNA are material expressions of that virtual code, initiated when an individual appears and develops.

The results presented here argue that time-related changes, ontogeny and phylogeny, share a common basis to be sought in the laws of thermodynamics, particularly in those pertaining to energy dynamics and the transformation of matter. We are critically pursuing this somewhat contentious point in current studies in Douglas fir (*Pseudotsuga menziesii*) and a grass, *Achnatherum extremeorientalis*.

### Acknowledgements

Cy Finnegan was instrumental in this paper, both as a source of ideas and clarification of the writing and arguments. RT acknowledges support from Natural Sciences and Research Council of Canada.



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