

**STUDY OF SOIL PROPERTIES
ALONG A HILLSLOPE
IN
PARSON'S PARCEL
BOULDER, CO**

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INTRODUCTION

Physical and chemical properties of soils can vary depending on the soils position in the local topography. The topography of an area can effect the microclimate, soil formation, parent material and hydrological and geological processes, which in turn effect soil processes (Birkeland 1984). Topographic factors such as the orientation of the hillslope and the steepness of the slope affect the microclimate, vegetation establishment, water movement, and erosion (Birkeland 1984).

Slope aspect has been shown to effect the temperature of the soil, vegetation establishment, and moisture levels. These factors in turn can affect the distribution of soil organic matter, the presence or absence or an E layer (in more humid areas), pH, and nutrient levels (Birkeland 1984). North facing slopes generally have less sunlight and in turn have higher moisture levels and greater vegetation establishment resulting in more organic matter. PH trends and nutrient levels are usually associated with vegetation and can also be affected by the slope aspect. Mesic vegetation types are usually found on the North facing slopes, while more xeric vegetation is found on the south facing slopes (Birkeland 1984).

Slope aspect is important in the development of soils, but the position of a soil on a slope has also been found to effect the properties of soil. The term soil catena is used to describe the lateral variation in soils over a hillslope (Young 1972, Birkeland 1984). Ritter (1986, pg. 96) defines a catena as "a group of soil profiles whose characteristics change gradually beneath a sloping surface." These changes in soil profiles are due to the variation of soil forming factors along the slope. Geomorphic processes such as the drainage of groundwater, transport of sediment and the removal of mobile

chemical elements are all important in the variation in soils over the slope (Ritter 1986).

The slope can be divided into sections which are commonly referred to as the ridge, crest, slope and toeslope (Figure #1.1). Steeper slope sections tend to be freely drained, while at the bottom of slopes (toeslope area) there is usually a higher moisture content due to poor drainage (Ritter 1986). Toeslope soils are not only higher in moisture content, but are also known to be richer in clay and organic matter (Birkeland 1984). In drying climates, saline soils are found in the less drained areas at the bottom, while ridge tops and crests undergo more leaching (Birkeland 1984).

In dry climates there is less moisture available and therefore in general there is less lateral transfer downslope than in a more humid region. A study in California found that the clay content of soils increase downslope, but most of this clay resulted from weathering of the underlying rock at the site and not lateral transport downslope (Birkeland 1984). The combination of increased clay and downslope movement of water in the rainy season result in an increase in the water holding capacity of the soil (Birkeland 1984). In other studies increases in iron oxides, pH and organic matter have been found downslope (Birkeland 1984).

In arid environments it has been found that only the more soluble compounds, such as salts, increase downslope, via lateral translocation. Calcium, magnesium, and sodium are found in the lowest parts of the landscapes because as compounds with chloride they are very soluble (Birkeland 1984). It is suggested that iron and aluminum released by weathering would stay at the site of release as they do not form very soluble compounds (Birkeland 1984).

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The purpose of this study was to examine the physical and chemical properties of a series of soil samples across a topographic gradient. The study site was a hillslope located in Parson's Parcel, in Boulder County Open Space. The transect measured approximately 240 meters in length and extended from one toeslope, up the slope across the ridge of the hillslope and down the other side to the opposite toeslope. The two slopes measure were approximately north and south facing. The annual rainfall in this area is 18.24 inches (Callahan 1986).

Several hypothesis were generated regarding the soil properties of the hillslope with respect to slope aspect and position on the slope. The north facing slope should have lower temperatures, different vegetation types and amounts, higher moisture levels and a greater quantity of organic matter than the south facing slopes. The chemical properties of the north slope should follow from the physical properties such that there would be an increase in pH and nutrient levels such as ammonium. The toeslope areas, regardless of aspect, should have a higher moisture content, be richer in clay and organic matter, and in turn have a higher water holding capacity than the upslope regions. The bulk density of these downslope regions should also decrease due to an increase in clay deposition and organic matter and the resulting increase in pore space. Chemically the pH and nutrient levels should also increase in the toeslope regions, regardless of aspect. Crest areas should be steeper, more eroded, have less organic matter, less clay, and less soil moisture.

2. METHODS

2.1 Sampling

All samples were collected on September 27/1993. Along the 240 meter transect there were 9 soil samples collected, approximately 30 meters apart. One sample was taken from the toeslope, crest and slope on each side of the hill slope. Three samples were taken from the ridge top (Figure #2.1). The samples were selected with the use of a random number table, within a ten meter section of the transect. Once the point was chosen, the samples were taken within a 25 cm radius of the point.

A soil auger was used to take the soil samples to a depth of approximately 15 cm. Twenty samples were taken within the 25cm radius of the point and placed in one bag and sealed to retain moisture. The large amount of soil was collected to ensure that the sample would be sufficient to complete all the laboratory analysis necessary. Two more soil samples of a known volume were taken at each point. Two 35.5 ml samples were taken for analysis where a known volume of soil is needed.

Observations at the site were made, including soil horizon descriptions of the first 15 cm. Slope angle and aspect were measured for all sample points. The temperature at a depth of 15 cm, the ground surface and at 1 m above the ground were measured and recorded. Litter depth values were recorded along with the % cover of the different vegetation types. The presence of rocks, crusty surfaces or drainage gullies were noted.

2.2 Laboratory Analysis

2.2.1 Moisture

Gravimetric soil moisture was calculated for each sample on an oven dry basis. 100 g samples were weighed and then oven dried at 110°C for 8 hours. The samples were then reweighed and the percent moisture was calculated using the formula;

$$(\text{original sample weight} - \text{dry weight}) / (\text{dry weight}) * 100$$

For each sample the air dry weight was also calculated using the above gravimetric formula. The oven dry weight subtracted from the air dry weight gives the weight of the hygroscopic water. This weight can be used to calculate the % hygroscopic water in the soil.

The water holding capacity (WHC) of the soil is calculated based on the weight of a known volume of soil that is saturated to the sticky point. The actual %WHC is calculated by the formula;

$$(\text{wt. of soil at sticky point} - \text{dry soil weight}) / (\text{dry soil weight}) * 100$$

The water holding capacity of the soil by volume can also be used to determine the % pore space (%PS) of the soil. The formula for the percent pore space is;

$$(\text{volume of water}) / (\text{volume of soil}) * 100$$

The gravimetric soil moisture, % hygroscopic moisture, % WHC, and the % PS were calculated for each sample along the transect.

2.2.2 Organic Matter

To determine the percentage of organic matter in each sample the loss-on-ignition method was used. The samples were placed in a muffle

furnace for 8 hours at 580°C. This ashes the organic matter in the soil. Therefore the difference between the weight of the sample before ashing and the weight of the soil following ashing is attributable to the loss of organic matter.

2.2.3 Soil Texture

Texture determination was done using the Bouyocous hydrometer method. The ashed soil samples were first ground in a 2 mm sieve to remove the coarse fraction of the soil. The percent coarse fraction was calculated as a portion of the weight of the total ashed weight. The remaining soil was considered the fine fraction.

The fine fraction was added to a sodium solution to deflocculate the individual soil particles. This solution was placed in a 1 liter cylinder and particle readings ^(in grams suspended solid per liter) were taken at 40 sec and 2 hours using a hydrometer. The percent sand, silt and clay were calculated based on these readings.

2.2.4 Bulk density

Bulk density is defined as the mass (weight) of a unit volume of dry soil. This volume includes both the pore space and the solid space. Using a known volume of soil the bulk density can be calculated by simply weighing the soil following oven drying to remove any capillary and hygroscopic water. This was done for all soil samples.

2.2.5 Soil pH

The pH of the soil samples were determined using both oven dried and air dried soils. Two different methods were used; 1:1 soil: water and

1:1 soil: CaCl₂. Therefore for each point four different pH analysis were preformed.

Equal weights of soil to weights of water or CaCl₂ were mixed and stirred and let to settle for approximately 1 minute. A pH meter was used to measure the values of pH in the solution.

2.2.6 Nutrients; NH₄ and SO₄

To determine the amounts of the nutrients NH₄⁺ and SO₄²⁻, first the soil samples underwent extraction procedures specific for each nutrient. To extract for NH₄⁺ a 1N KCl solution was used to mass displace the NH₄ in the soil sample. A solution of ammonium-acetate/acetic acid was used to extract for SO₄²⁻ in the soil sample.

To measure ammonium the extractant is mixed with a number of reagents including phenate. The phenate reacts with the other reagents and the ammonium in the solution to form a blue colored solution that reflects the amount of ammonium. The solution was placed into the spectrophotometer at 630 nm and the absorbance was read. This absorbance was then compared to a standardized curve and the meq/liter of ammonium in the soil sample was determined. To convert this to meq/100 g of soil the meq/liter was simply multiplied by the dilution factor, which in this case was 0.4.

To measure sulfate the same procedure was used as was for ammonium except there were different reagents needed. The reagents for sulfate included BaCl₂ which forms BaSO₄, if SO₄ is present. BaSO₄ is a precipitate and the absorbance of the precipitate was measured in the spectrophotometer. To determine the meq/100 grams of soil, the same procedure was used as for ammonium, except the dilution factor was 0.25.

2.3 Numerical Analysis

A series of line graphs were created to show the variation of the different soil properties over the hillslope. Properties such as pH, texture, nutrient concentrations, % moisture, % bare ground, % organic matter, A horizon depth, temperature, and bulk density, were all graphically represented.

For the north and south facing slopes averages of the different soil properties were calculated to look at the effect of aspect. Averages were also calculated for the two toeslopes, mid-slopes, crests, and the three ridge sites to examine the the effect of slope position.

Regression analysis was performed on a number of variables to see if certain soil properties were associated with one another. Only regressions with a probability of less than 0.1 were considered significant, with a emphasis on the significance of associations with a p value of less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Slope aspect

The first set of hypothesis^s were related to variation in physical and chemical soil properties according to the aspect of the slope sampled. Many studies have suggested that the north facing slope should have lower temperatures, different types of vegetation, more vegetation cover, higher moisture levels and a greater quantity of organic matter than the south facing slopes. The chemical properties of the north slope should follow from the physical properties such that there would be a decrease in pH and an increase in nutrient levels such as ammonium.

The two slopes sampled were approximately north and south facing with a compass heading of approximately 16° for the north facing slope and 145° for the South facing slope. The south facing slope on average was not as steep as the north facing slope with angles of 8.67° and 13.67° and the mesa top (or ridge top) was relatively flat (Table 3.1)

As predicted the south facing slope ^{soil temperature} on average was warmer than the north facing slope, while the mesa was the warmest. The north facing slope on average was over 3°C cooler than the south facing slope (Table 3.1, Figure 3.1). Ground temperature on the two slopes were both 20°C on average, while the air temperature on the north facing slope was actually just over a degree warmer than the south facing slope (Table 3.1, Figure 3.1). This could be due to differences in the time the air temperatures were measured or the fact that air temperature fluctuates more than soil or surface temperatures due to wind effects. The soil temperature is a better and more steady indicator of diurnal temperature fluctuations.

Unlike the predictions of the hypothesis, the A horizon depth does not seem to vary according to slope. Both slopes have an average A horizon depth of 3.5 cm. The mesa top actually has the narrowest A horizon with an average depth of only 2 cm (Table 3.1). The deeper A horizons along the slopes are probably due to the over-representation of A horizon depth in the average, by the toeslope values (Figure 3.2). Figure 3.2 shows that the lowest A horizon depths are found at the the crest sampling points, while the highest values are the toeslopes.

Percent bare ground values are actually opposite to what one would expect given the fact that south facing slopes are usually drier. The south facing slope in this study showed less bare ground than did the north facing slopes with an average of 18.7 % and 41.7% respectively (Table 3.1). This

could be due in part to the different species of plants that occupy the two slopes. Since the area is fairly arid most of the plant species may be more xeric in nature and therefore may actually prefer the south facing slopes. The observations made by the individuals at each point show that at the toeslope of the north facing slope the sample point was adjacent to a gully. This could result in a lack of vegetation establishment in this area. Therefore the lack of vegetation on the north facing slope could reflect the effect of erosion and drainage rather than the topographic aspect.

Soil moisture content is on average slightly higher on the north facing slope at 17.39 %, but the south facing slope is not much drier at 17.19%. The mesa top actually has the highest moisture content at 20.38 % (Table 3.1). The observations show the mesa top to not be as rocky as the sides and to have lots of grass cover. Therefore, it follows that this area would not experience as much evaporation as the slopes, resulting in the higher moisture in the soil. The ridge or mesa top is probably the best drained area following a rainfall event, but given average conditions, at least several days following the last rain event, the ridgetop probably retains capillary and hygroscopic water. Figure 3.3 shows both % moisture and % hygroscopic water are highest along the ridgetop. Percent hygroscopic water is actually highest on the south facing slopes at 2.8%, while the north facing slope has only 2.36 % hygroscopic water on average (Table 3.1).

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The water holding capacity of a soil affects the depth of infiltration of water and is dependent upon the textural makeup of the soil, the compaction of the soil, and the amount of organic matter in the soil. The water holding capacity for this hillslope is greater on the south facing slope than on the north facing slope, which is counter to the hypothesis in the first section. The water holding capacity on average is 31.12 % on the south

facing slope while it is 29.68 % along the north facing slope (Table 3.1, Figure 3.4). Since water holding capacity of the south facing slope is greater it should follow that the percent organic matter is also greater along the south facing slope. The percent organic matter along the south facing slope is 7.22%, while it is only 5.91 % along the north facing slope (Table 3.1, Figure 3.5, Figure 3.6). A regression showed a significant ($p=0.05$) and positive (slope= 12.8) relationship between % organic matter for all sites and the % WHC by weight for all sites (See Appendix, figure 5 for scattergram).

Four methods of determining pH were used for all soil samples. Regression analysis showed that the pH for the oven dry and air dry samples that were both tested with the 1:1 water method were significantly associated ($p=0.0009$) in a positive manner. The oven dry and air dry samples that were tested with the 1:1 CaCl_2 were also significantly ($p=0.007$) related in a positive association. This is to be expected but the test regression analysis helped to show the precision in these results as they were repeatable within the method, no matter if they were air dried or oven dried. As expected the oven dry samples for both the 1:1 water and the 1:1 CaCl_2 methods, were lower than the air dry samples in the respective methods (Table 3.1, Table 3.2, Figure 3.7 and Figure 3.8).

The hypothesis suggests that pH should decrease along the north facing slope due to increased moisture and greater vegetation cover. But the results so far have shown that moisture and vegetation cover did not increase along the north facing slope, therefore it should follow that pH will not agree with the hypothesis. The south facing slope does in fact have lower pH values for all methods used (Table 3.1) along the south facing slope compared to the north facing slopes, counter to the original

hypothesis. This discrepancy could be a result of higher moisture and greater vegetation cover along the south facing slope for reasons already discussed.

Nutrient levels were hypothesized to increase along the north facing slope. NH_4 does show higher levels of concentration along the north facing slope, with an average value of 0.51 meq/100 g soil, while the value for the south facing slope is only 0.29 meq/100 g soil (Table 3.1, Figure 3.9). The values for ammonium correspond in a positive fashion to the values for the depth of the A horizon. A regression shows a significant association ($p=.02$) between the two variables (See Appendix A, figure 1 for scattergram). It is not surprising to find ammonium associated with the organic layer in the soil, but what is surprising is not to find a positive relationship between the organic matter in the soil and the depth of the A horizon. In fact, regression analysis showed a significantly ($p=.07$) negative relationship between the two (Figure 3.6, and see Appendix A, figure 7 for scattergram). This could be a result of the different sampling methods.

The determination of the A horizon was a visual estimate made in the field. The organic matter determination was made in the lab using only 100 grams of the soil from the entire sample collected. If the sample as a whole under-represents organic matter because of the depth of sampling, one would see a discrepancy in the relationship between the depth of the A horizon and the % organic matter. If we had all sampled to the exact same depth at each site, one would expect a significantly positive relationship between the % organic matter in the sample and the depth of the A horizon. The site at which I sampled was rocky and as a result it was very hard to dig to the 15 cm. If some people did get to 15 cm and others did not, then the soil samples would vary in their accurate representation of organic matter.

Sulfate concentration was greater along the south facing slope than along the north facing slope with an average of 0.22 meq/100 g soil and .06 meq/100g soil, respectively. Regression analysis showed Sulfate to be significantly correlated with both % organic matter ($p=.03$) and % WHC ($p=.1$), with both relationships such that a rise in either % organic matter or % WHC were associated with a rise in the concentration of sulfate (See Appendix A, figures 4 and 6 for scattergrams).

3.2 Soil Position along slope

The second set of hypothesis were related to variation in physical and chemical soil properties according to the position of the soil on the slope sampled. The toeslope areas, regardless of aspect, should have a higher moisture content, be richer in clay and organic matter, and in turn have a higher water holding capacity than the upslope regions. The bulk density of these downslope regions should also decrease due to clay deposition and increased organic matter. Chemically the pH and nutrient levels should also increase in the toeslope regions, regardless of aspect. Crest areas should be steeper, more eroded, have less organic matter, less clay, and less soil moisture.

According to the above hypothesis Organic matter, and the depth of the A horizon should increase as one moves downslope. Table 3.2 shows that the depth of the A horizon does indeed increase towards the toeslope, with a value of 1 cm at the crest and increasing to 3 cm at the mid-slope and 6.5 cm at the bottom. It should follow that the percent organic matter in the soil should also increase towards the bottom of the slope due to the downward translocation of plant litter and other organic matter. The organic matter in the soil sample actually is highest mid-slope and the

toeslope has a value only slightly higher than the crest (Table 3.2). The ridgetop has the highest average % organic matter with a value of 8%, which is probably due to the flatness of the area, the low amount of annual rainfall and the grass cover. In a more humid region this area would be highly leached, but in this arid environment organic matter is allowed to accumulate over time.

The discrepancy between the values for % organic matter and the depth of the A horizon are clearly shown in figure 3.6. It shows that the greatest difference is in the toeslope areas. Both Observations show very deep A horizons, but very low values for percent organic matter. It may be that these two samples were over-represented by mineral soil due to the depth of sampling, reflecting a general problem with composite sampling even at one point.

The steepness of the slope section should affect the movement of water along the hillslope, resulting in patterns of erosion, vegetation establishment, soil moisture and pH. The midslope regions of this hillslope were on average the steepest at an angle of 14.5° . The two crest slopes were very very different with the north facing crest at an angle of 21° and the south facing crest was only 6° . As expected the percent moisture in the soil significantly ($p=.05$) increased as slope angle decreased (See Appendix A, figure 11, for scattergram).

The original hypothesis suggested that pH should increase in the toeslope regions due to an increase in organic matter and soil moisture. Analysis showed the opposite to be true in this study; as slope angle increased there was a significant ($p=.008$) increase in pH. This may be due to the fact that soluble salts such as chlorides of calcium and magnesium are transported downslope along with moisture (Birkeland 1984) (See

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Appendix A, Figure 11, for scattergram). PH was also found to decrease significantly ($p=.04$) as temperature increased. This could be due to increased productivity given the right moisture conditions.

Observations by the individual samplers, in regards to vegetation, did not show any distinct difference in vegetation from one position on the slope to the next. Almost all the observers noted grass in their sampling plot, and many of them recorded the occurrence of xeric species such as yucca, cactus and sage. Most of the sites were rocky or recorded gravel and one site was even described as "way rocky". Percent Bare ground did not show a pattern according to slope position, with the ridgetops and the mid-slopes having the least bare ground, while the crest sites and the toeslopes had 10-15% more bare ground on average (Table 3.2). The variation in bare ground percentages may reflect the sampling error by the individual. If only one person had made the measurement at each site then the estimates may be more precise. As a group we did not calibrate our measuring estimates, therefore a difference of 10-15% may be attributable to the way different individuals perceive the percentage cover.

Since organic matter (and depth of A horizon) is hypothesized to increase downslope, it follows that the water holding capacity and pore space should increase. The organic matter content of the soil was not found to increase downslope, due to sampling problems already mentioned, but the observed depth of the A horizon did increase downslope (Table 3.2).

Water holding capacity and pore space both decrease downslope, which is opposite to the hypothesis (Table 3.2, Figure 3.4). It follows then, counter to the hypothesis, that the relationship between %WHC and the depth of the A horizon is a negative one, such that the %WHC capacity

decreases significantly ($p=.07$) as A horizon depth increases (See Appendix A, figure 2, for scattergram).

The relationship between organic matter content of the soil and percent water holding capacity is probably a more comparable one, since they were both taken from the same sample, unlike A horizon depth which was from visual observation. Regression analysis did find a significant ($p=.05$) and positive relationship between water holding capacity and organic matter in the samples. Both water holding capacity and organic matter tended to decrease downslope, probably due to an over-representation of mineral soil in the downslope samples (deeper samples).

Pore space in the soils mirrored the %WHC fairly well decreasing from 32.89 % at the crest to 29.63% at the toeslope (Table 3.2, Figure 3.4). The original hypothesis was that the bulk density should decrease downslope due to increased organic matter, clay deposition and the resulting increase in pore space. The relationships between bulk density and the organic matter content along with the water holding capacity of the soil do follow the expected patterns. As organic matter content of the soil increases there is a highly significant ($p=.005$) drop in bulk density (See Appendix A, Figure 8, for scattergram). As bulk density of the soil increases the water holding capacity of the soil significantly decreases ($p=.03$) (See Appendix A, Figure 9, for scattergram).

The bulk density of the soil is the weight of the soil per unit volume and it describes the density of the soil solids compared to pore space. Since neither the water holding capacity of the soil or the pore space increase downslope, the bulk density should increase in a downslope manner, as a result of a decrease in pore space (Figure 3.12). The data does show an

increase in bulk density from 0.83 at the crest to 1.11 at the toeslope (Table 3.2, Figure 3.11).

The increase in bulk density and the decrease in pore space may in part be due to the translocation of clay. Usually an increase in clay is associated with a decrease in bulk density. But if the clay particles are translocated downslope with water, they may be deposited in such a manner as to line the pores between coarser material, in turn decreasing pore space. The observation on the north facing toeslope did note that their point was next to a drainage gully indicating an area of increased water flow at certain rainy or melt times of the year. These areas may have increased clay contents, but not deposited in such a way as to increase pore space. Some studies have also suggested that in arid regions much of the clay formation may not be due to the translocation of weathered materials, but instead from in-situ weathering (Birkeland 1984, Brady 1990).

The clay content of the soil is seen to increase drastically from the crest at 2.9 % to the toeslope at 10.66% (Table 3.2, Figure 3.10). The coarse fraction of the soil and the percent sand are both lower in the toeslope areas than at the crests (Table 3.2).

Nutrients are hypothesized to accumulate downslope in conjunction with water movement and organic matter deposition. Sulfate is a soluble anion that in solution is readily available for plants (Brady 1990). In arid regions sulfate is known to accumulate in the lower horizons of mollisols, but also to accumulate on the surface in more saline conditions (Brady 1990). In this study sulfate was found to decrease downslope (Table 3.2), but also to be significantly ($p=.03$) related to the organic matter content of the soil (See appendix A, figure 4, for scattergram). Since organic matter does

not increase downslope, it follows that sulfate would decrease in a downslope manner also.

Ammonium increases drastically in a downslope manner in this study, from 0.18 meq/100 g soil at the crest to 0.68 meq/100 g soil at the toeslope. Regression analysis found the depth of the A horizon and the concentration of ammonium to be significantly ($p=.02$) and positively associated. This relationship may be a spurious one as a more comparable relationship would be between ammonium and % organic matter, which in this study would be opposite to the hypothesis. What may be driving this drastic increase in ammonium downslope is the relatively high value of 1.1 meq/100 g soil for the north facing toeslope. What is interesting to note is that this is the only site for which no comments were made regarding vegetation. The bare ground at this site is only 30%, but as mentioned before estimates of bare ground are very subjective. It could be that this site has the least quantity of biomass and therefore the least amount of nitrogen ~~fixation~~^{use} by plants. Since we did not take measurements to verify this, it remains a hypothesis.

4. CONCLUDING REMARKS

In general this study site did not mirror the hypothesized ideal of soil properties for a hillslope catena. This may be due to the fact that each point was sampled by a different researcher and therefore the precision of some of the measurements may be called into question, especially the visual estimations, of ground cover and A horizon depth. The depth to which each individual was able to sample at each point also varied, adding to over and under representation of some of the soil properties such as % organic matter and textural components (sand, silt, clay). Keeping the A and B

horizon samples separate for analysis would have shed some light on this problem.

Several properties of the soil were not measured such as nutrient concentrations of calcium and magnesium. It would have been very interesting to examine the base saturation of these soils. Sodium was not measured and it would have been interesting to see how the sodium levels in the soils changed over the hillslope, and how they related to the ph of the soils.

One important factor mentioned in several texts (Birkeland 1984, Ritter 1986, Brady 1990) is the importance of parent material on the soil forming processes and the resulting soil properties. We did not study the parent material of the area or observe whether or not the parent material on one part of the slope differed from another. Any discontinuity in parent material may account for some of the anomalous finding in our study.

there was a pin change from ridge to valley

As mentioned throughout the paper, vegetation play a very important role in soil formation and development. Any study of soil properties should take very careful account of the vegetation above the sample point and the same can be said for vegetation scientists regarding soil. Our study did make note of vegetation, but a more thorough study of the vegetation of the area in conjunction with certain soil properties would be interesting.

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Figure 1.1

Schematic Diagram of Slope Descriptors

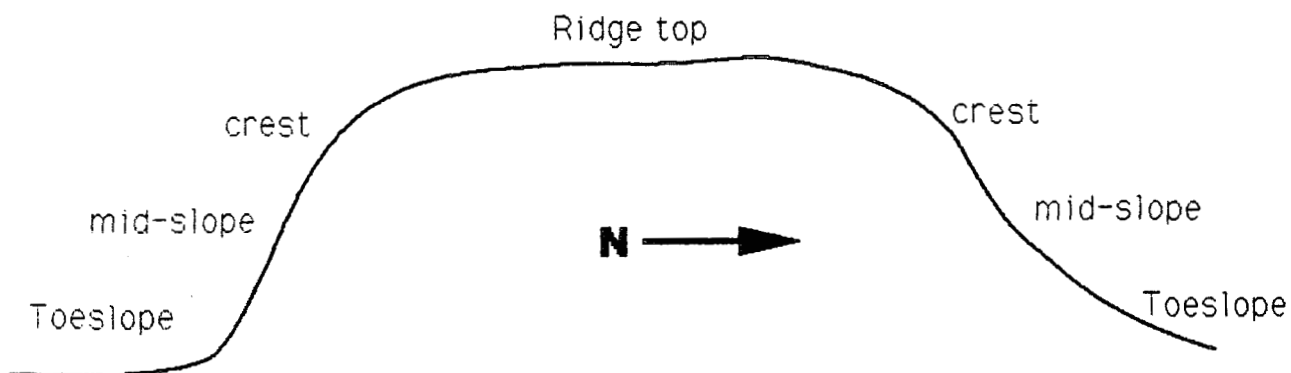


Figure 2.1

Sample sites, Bag Numbers and Person who sampled

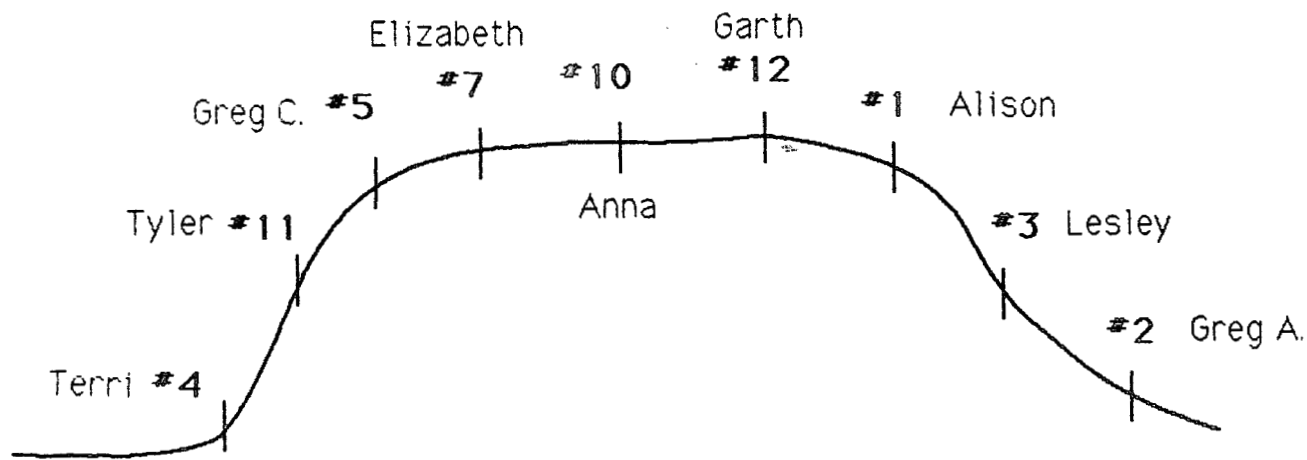


Table 3.1 Average values for soil properties according to slope aspect

Soil Property	South facing slope	Mesa top	North facing slope
Slope angle	8.67	1.83	13.67
soil temperature	6.17	7	3
ground temperature	20	14.83	20
air temperature	18.67	16.67	20
A horizon depth	3.5	2	3.5
% bare ground	35	30	41.67
% moisture (ovendry)	17.19	20.38	17.39
% hygroscopic water	2.8	3.81	2.36
% WHC	31.12	36.86	29.68
% Pore space	29.99	30.59	32.04
pH 1:1 (airdry & water)	7.16	6.13	7.42
pH 1:1 (airdry & CaCl ₂)	6.12	7.16	7.68
pH 1:1 (ovendry & water)	5.45	5.22	5.84
pH 1:1 (ovendry & CaCl ₂)	4.95	5.5	5.93
NH ₄ meq/100 g soil	0.29	0.15	0.51
SO ₄ meq/100 g soil	0.22	0.22	0.06
% Organic matter	7.22	8	5.91
Bulk Density	0.95	0.81	1
% coarse fraction	13.1	9.26	11.36
% sand	83.36	68.15	68.54
% silt	13.95	20.18	18.02
% clay	2.69	11.66	13.45

Figure 3.1

Soil, Ground and Air Temperature



Figure 3.2

A Horizon Depth and % Bare Ground for all samples.

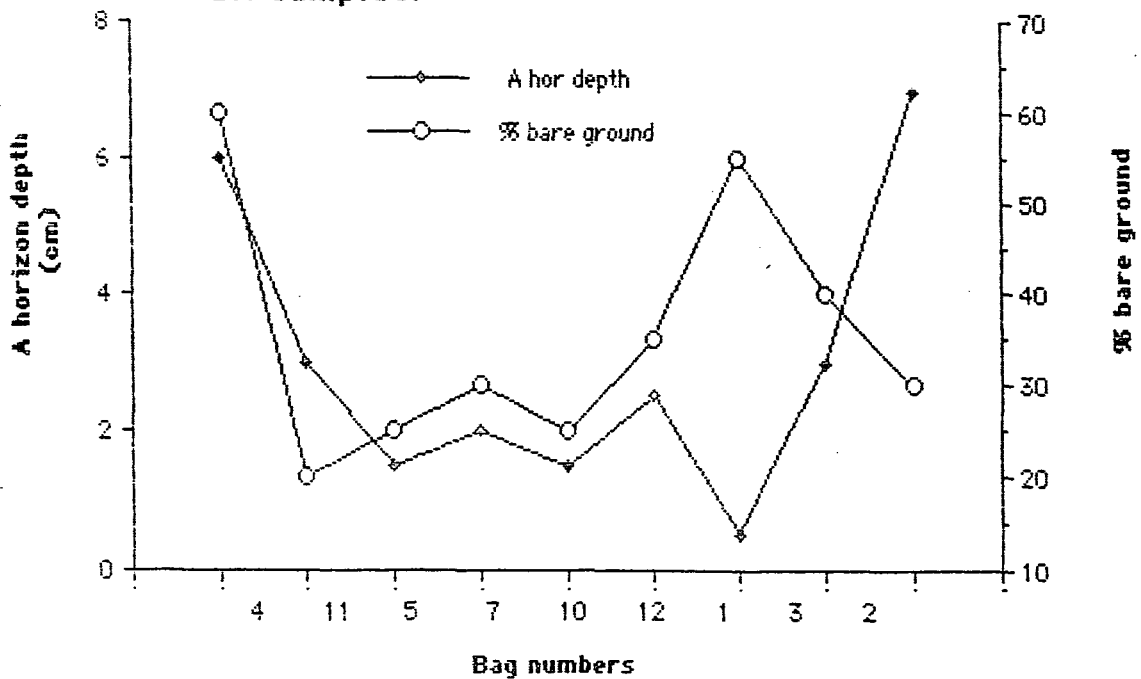


Figure 3.3

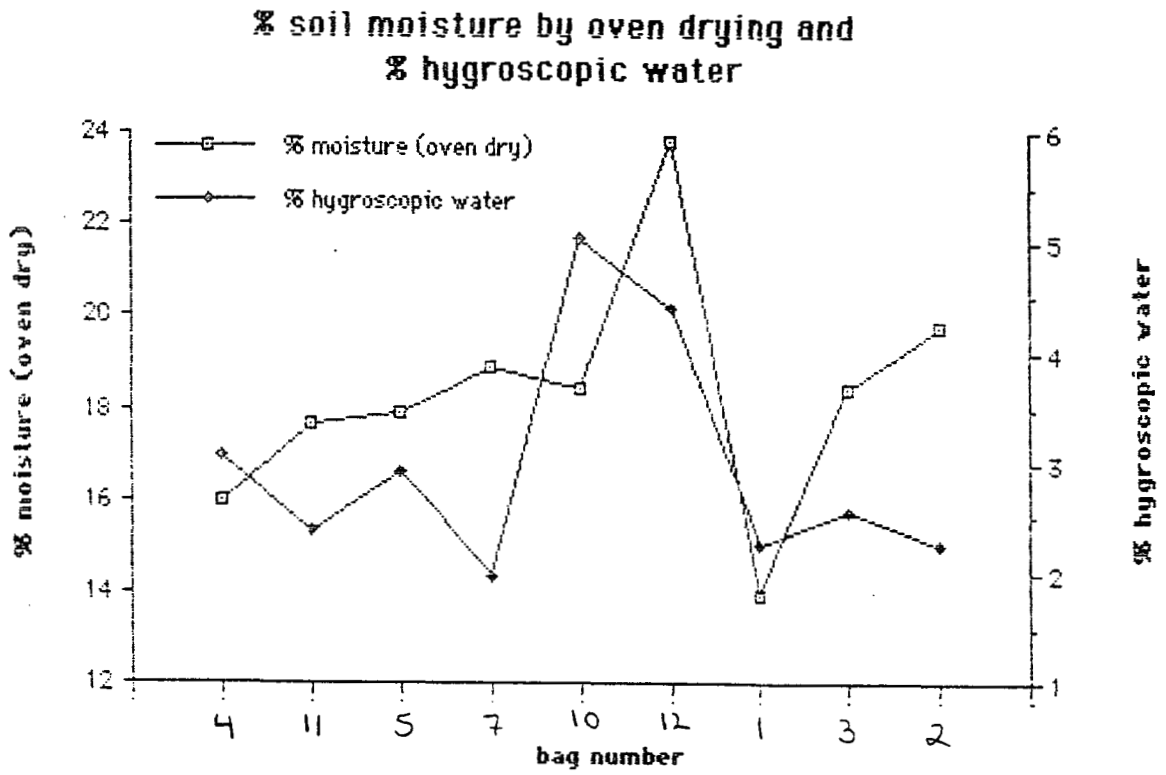


Figure 3.4

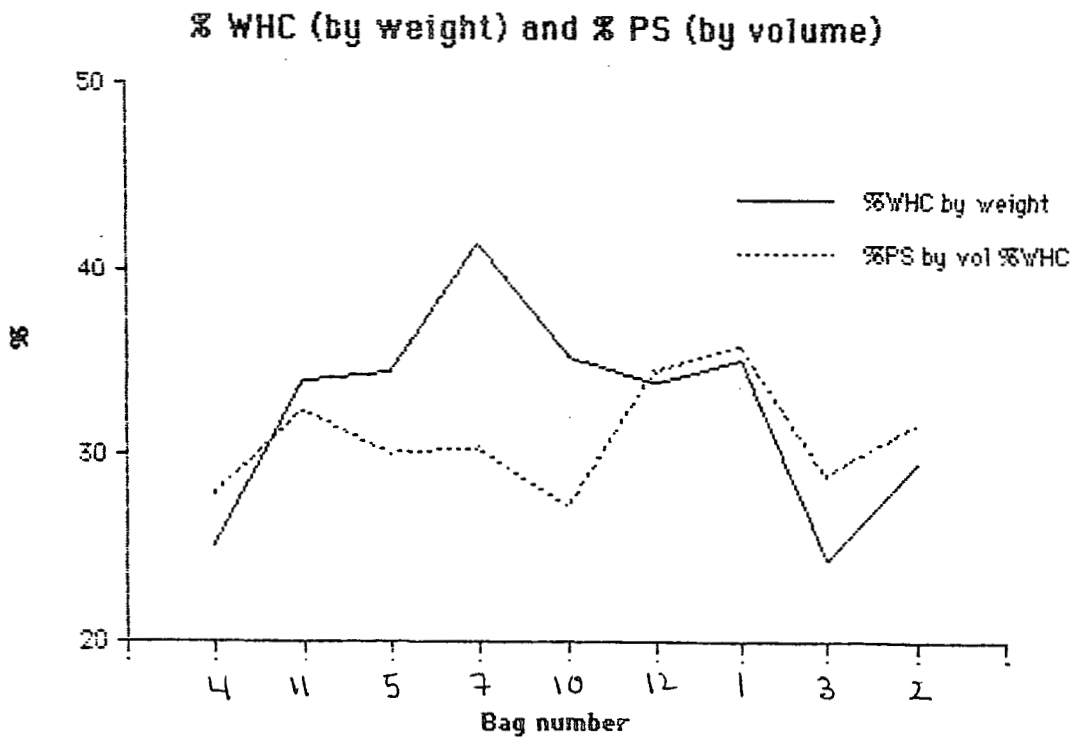


Figure 3.5

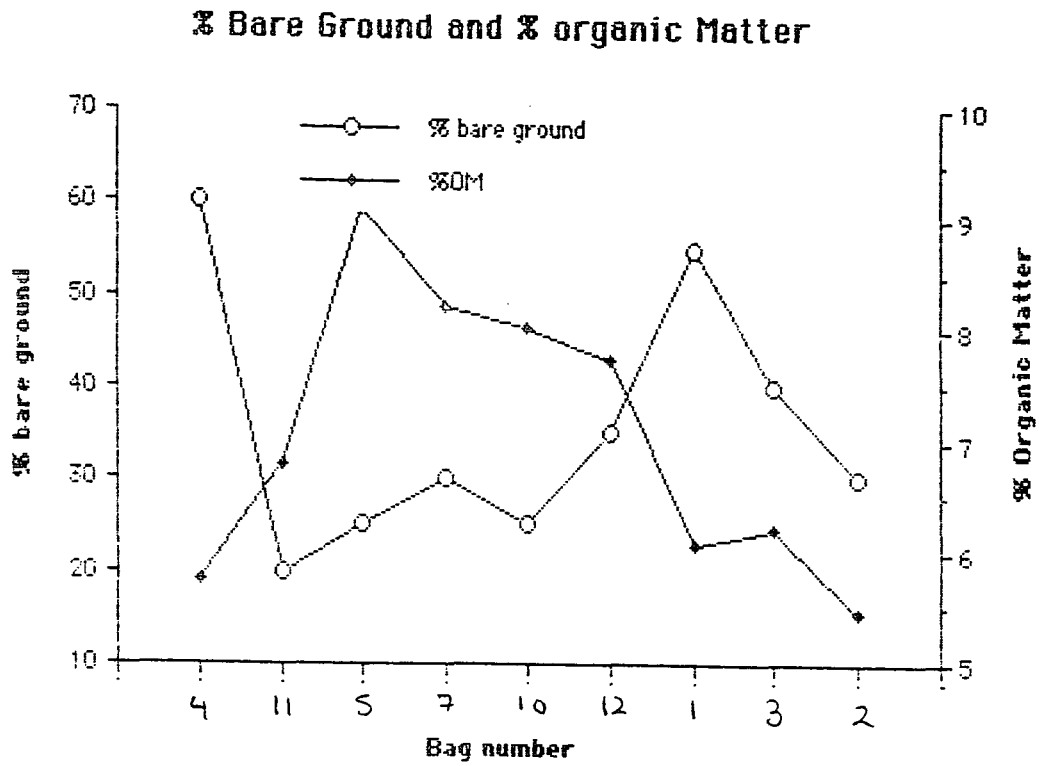


Figure 3.6

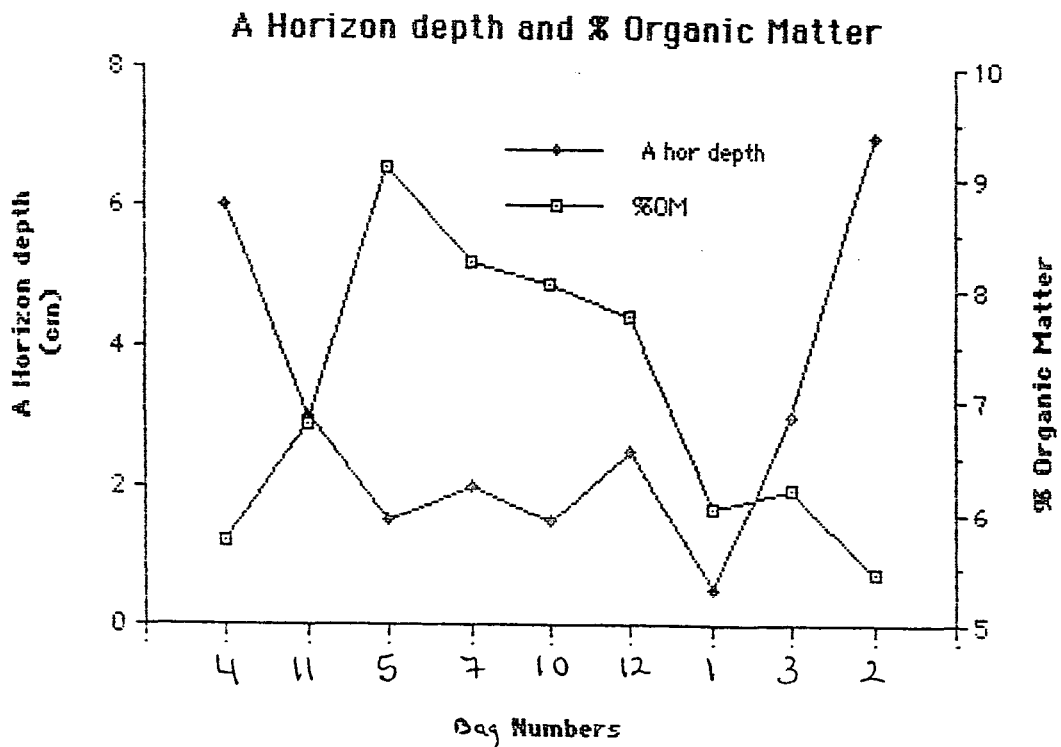


Figure 3.7

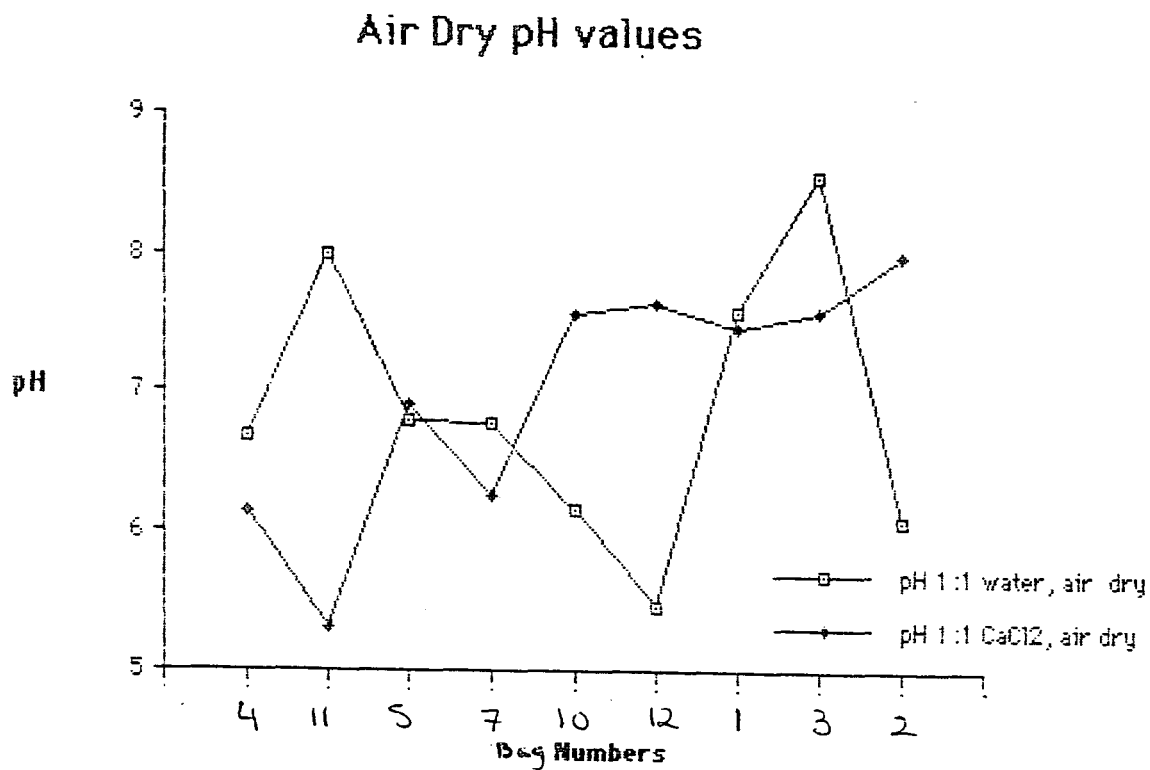


Figure 3.8

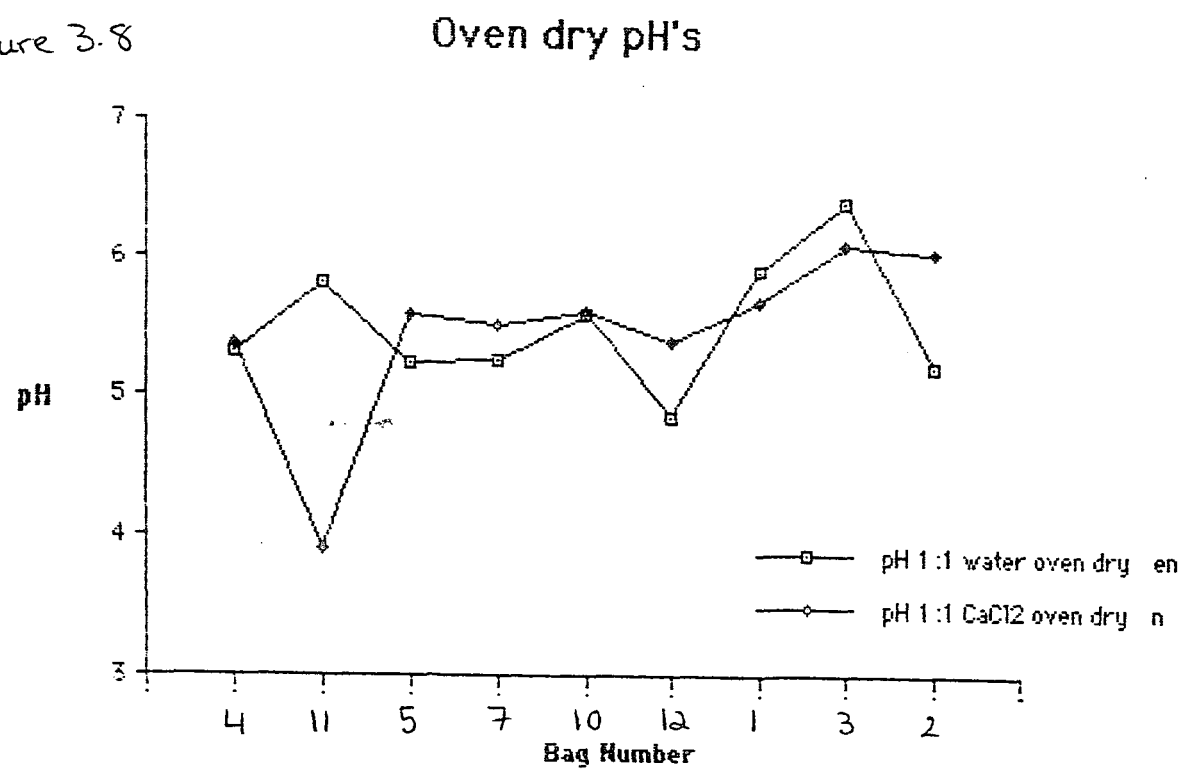


Figure 3.9

Concentration of NH₄ and SO₄ in the soil samples

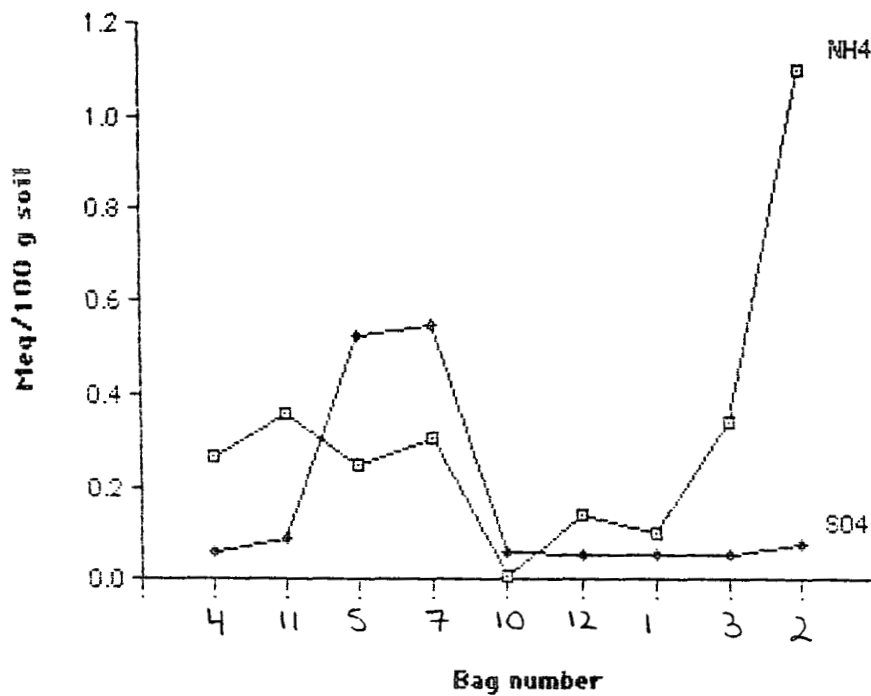


Figure 3.10

Textural composition of the soil samples

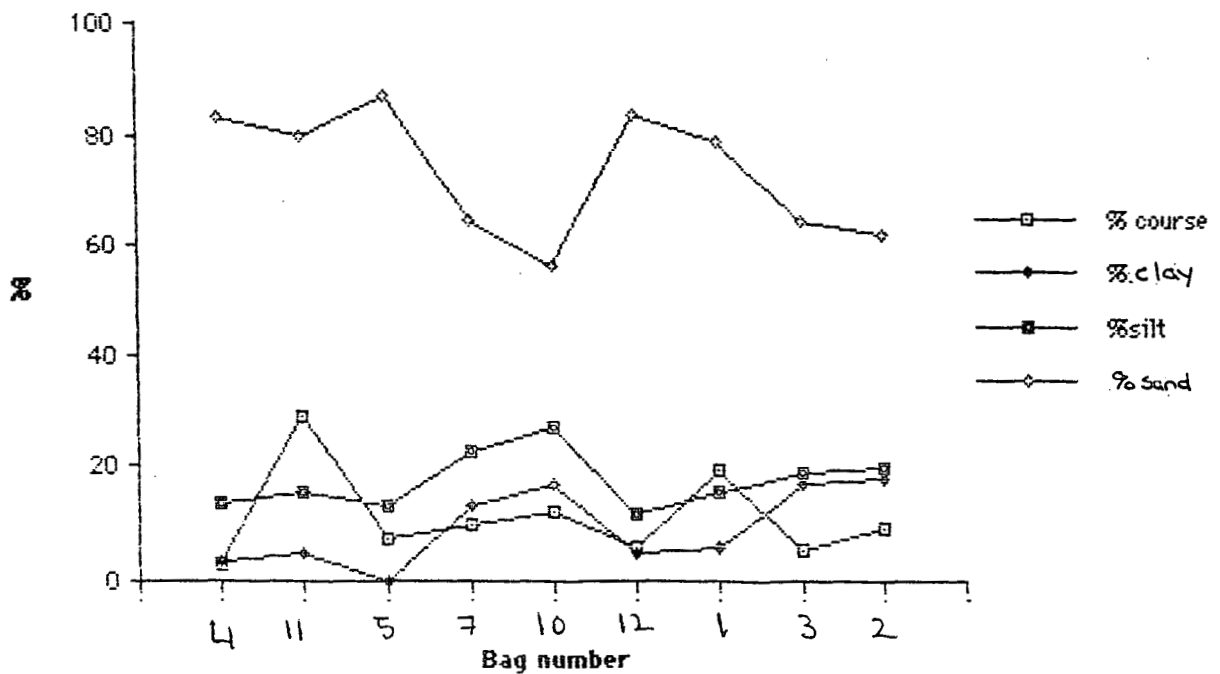


Table 3.2

**Average values for soil properties
according to the slope position**

Soil Property	Ridge top	Crest	Mid-slope	Toeslope
Slope angle	1.83	13.5	14.5	5.5
soil temperature	7	4.25	3.5	6
ground temperature	14.83	21.5	19	19.5
air temperature	16.67	19.25	19.5	19.25
A horizon depth	2	1	3	6.5
% bare ground	30	40	30	45
% moisture (ovendry)	20.38	15.94	18.06	17.88
% hygroscopic water	3.81	2.6	2.47	2.68
% WHC	36.86	34.8	29.15	27.26
% Pore space	30.59	32.89	30.53	29.63
pH 1:1 (airdry & water)	6.13	7.19	8.29	6.38
pH 1:1 (airdry & CaCl ₂)	7.16	7.2	6.45	7.07
pH 1:1 (ovendry & water)	5.22	5.57	6.1	5.26
pH 1:1 (ovendry & CaCl ₂)	5.5	5.63	5	5.7
NH ₄ meq/100 g soil	0.15	0.18	0.35	0.68
SO ₄ meq/100 g soil	0.22	0.29	0.07	0.07
% Organic matter	8	4.58	6.51	5.6
Bulk Density	0.81	0.83	0.99	1.11
% coarse fraction	9.26	13.41	17.11	6.18
% sand	68.15	83.02	72.2	72.63
% silt	20.18	14.09	17.15	16.72
% clay	11.66	2.9	10.66	10.66

Figure 3.11

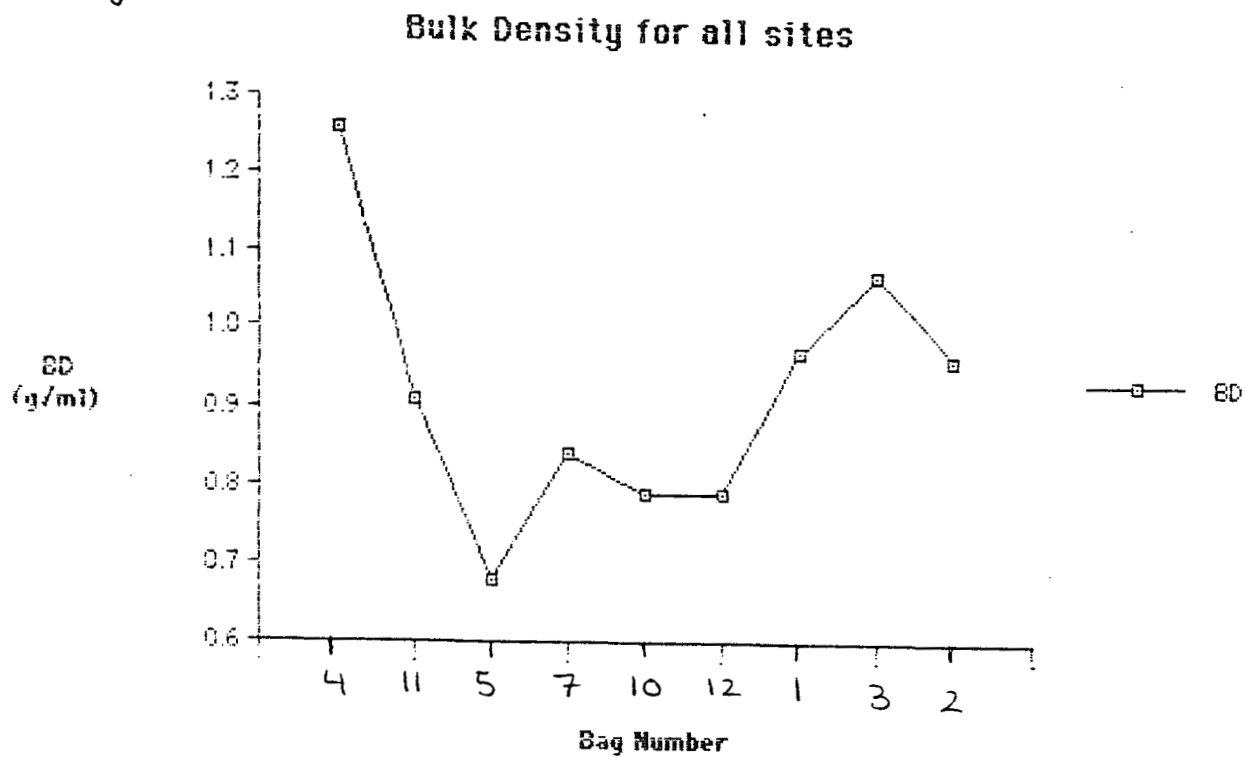
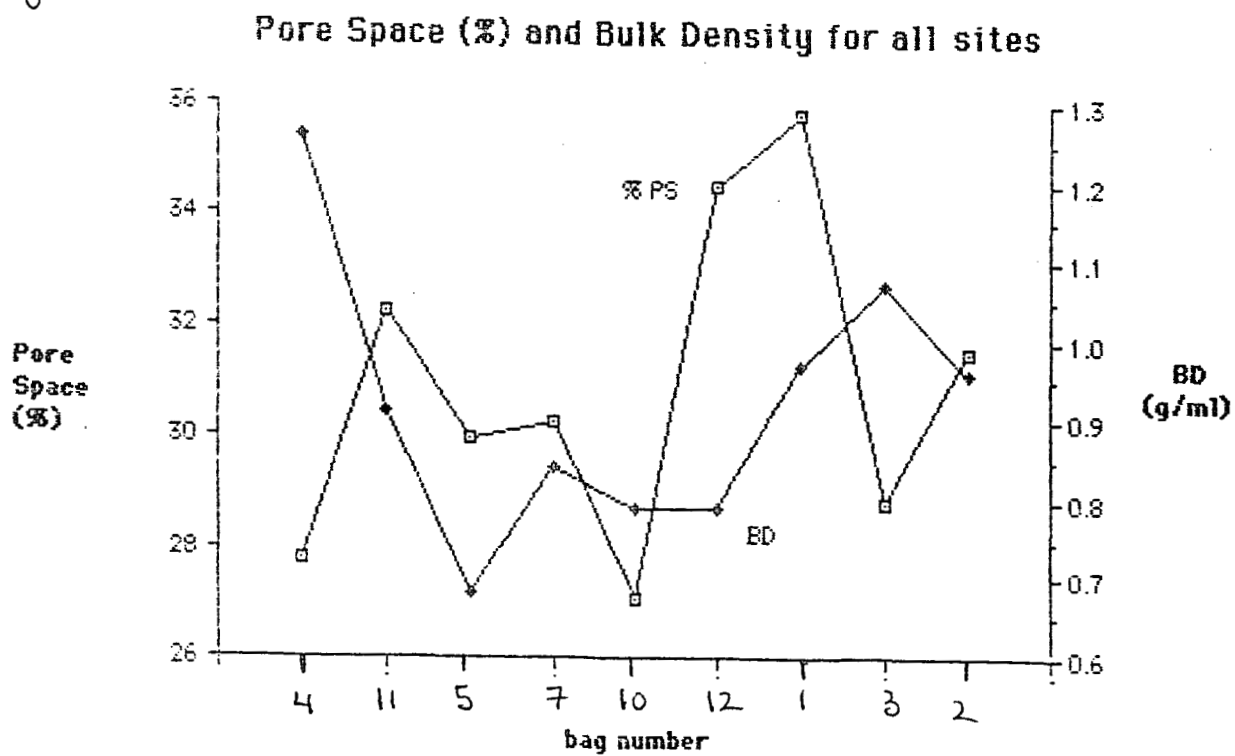


Figure 3.12



Appendix A

Regression Analysis of Associations Between Soil Properties

Figure 1

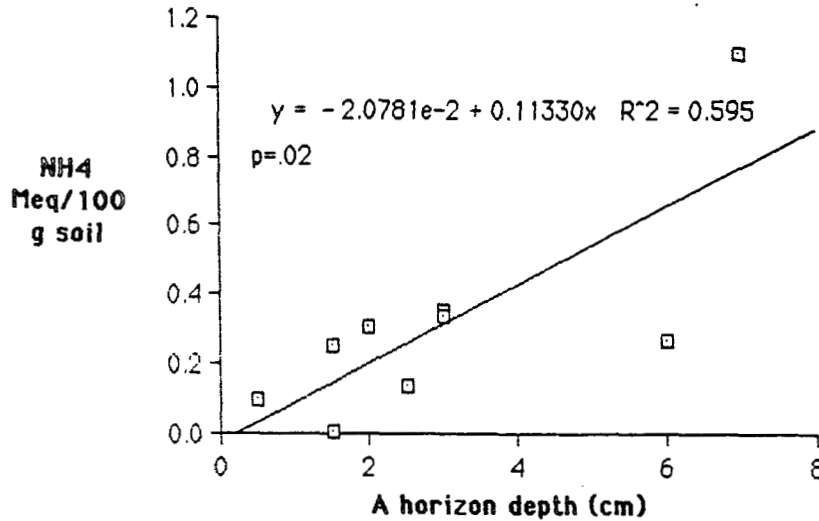


Figure 2

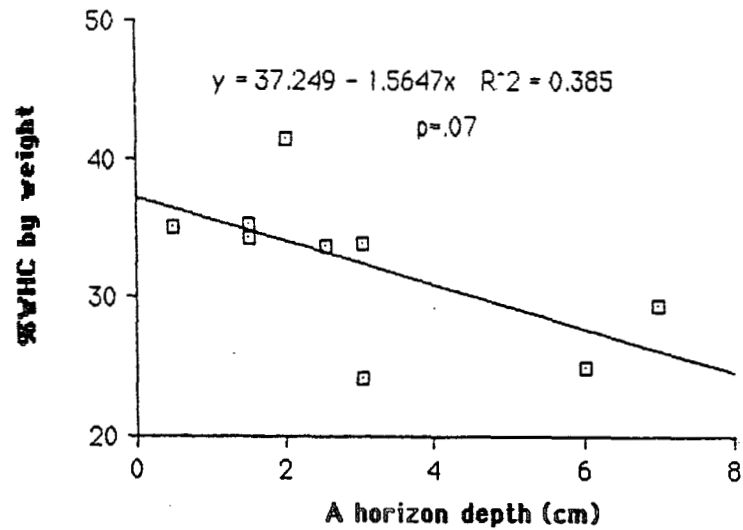
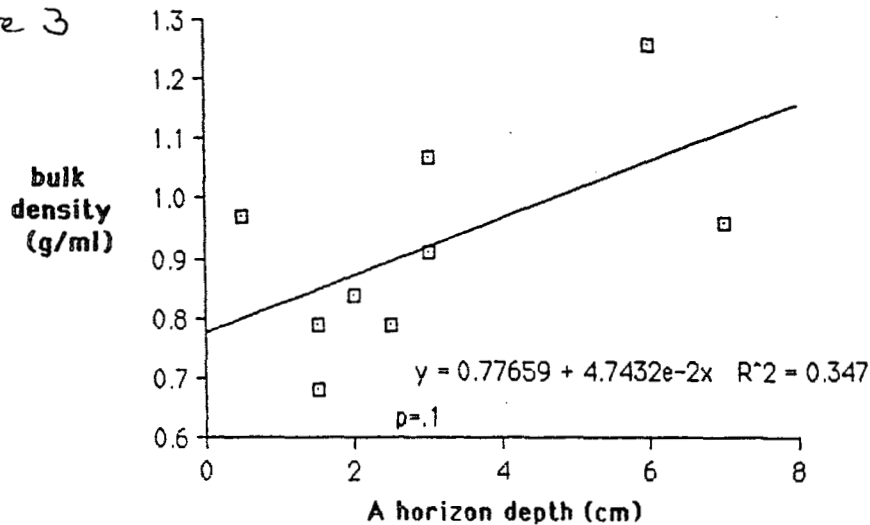


Figure 3



Appendix A

Regression Analysis of Associations Between Soil Properties

Figure 4

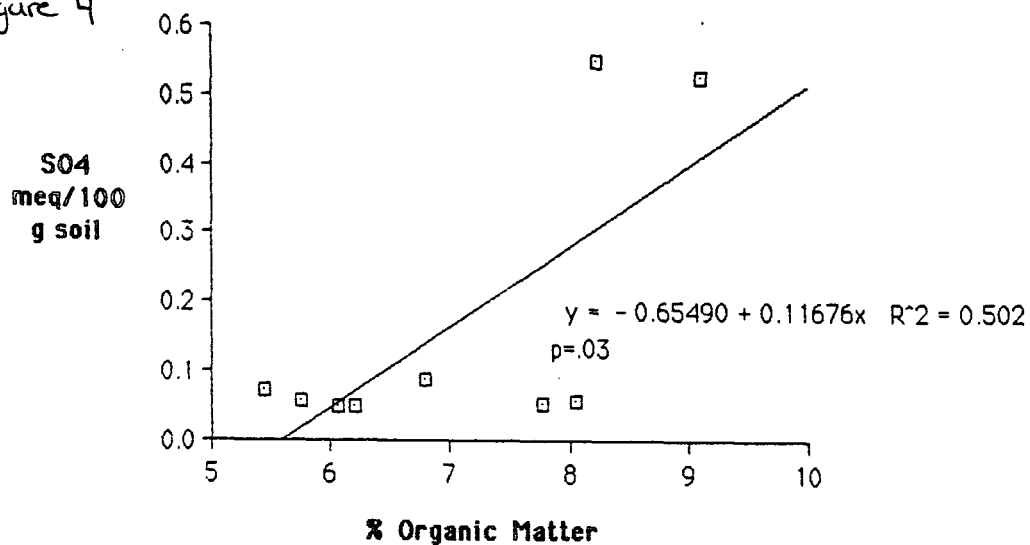


Figure 5

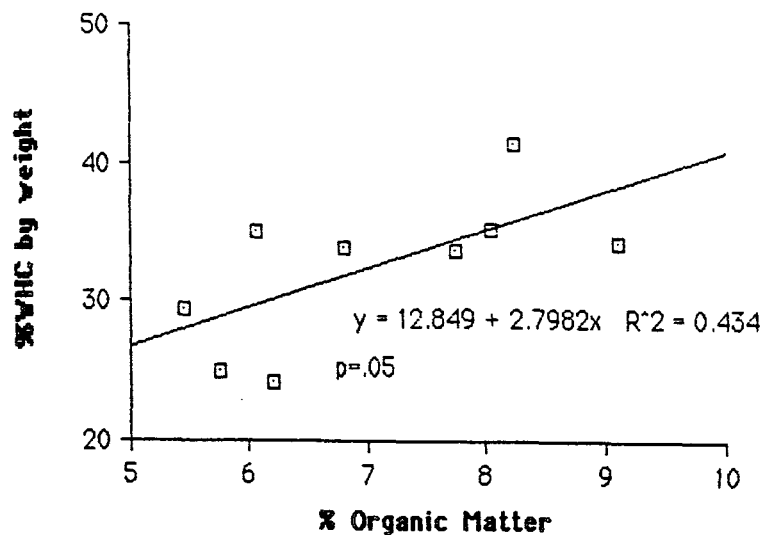
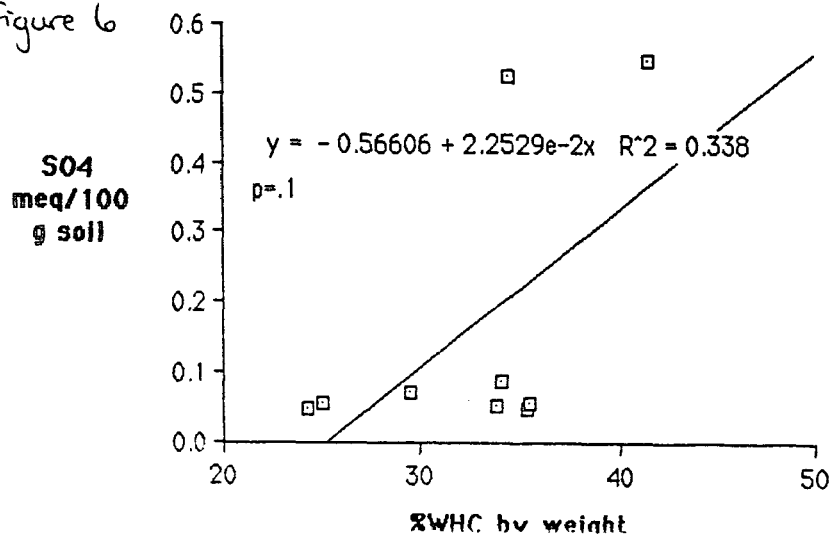


Figure 6



Appendix A

Regression Analysis of Associations Between Soil Properties

Figure 7

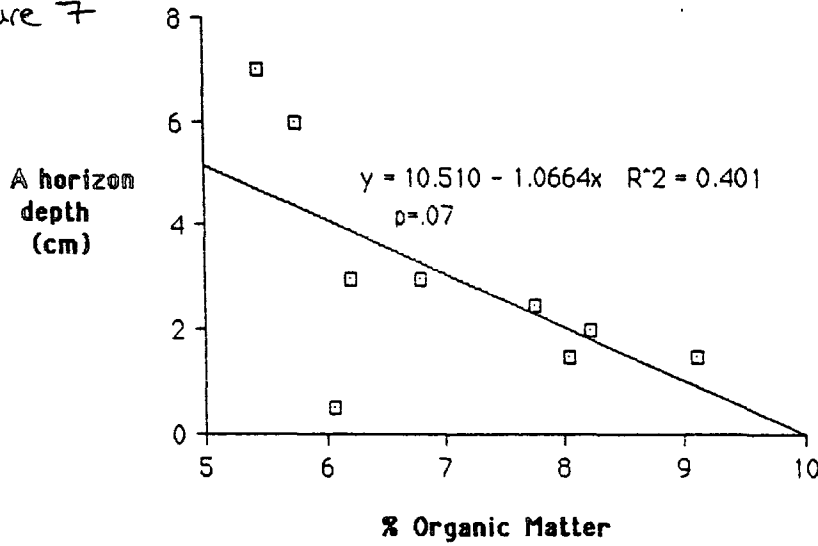


Figure 8

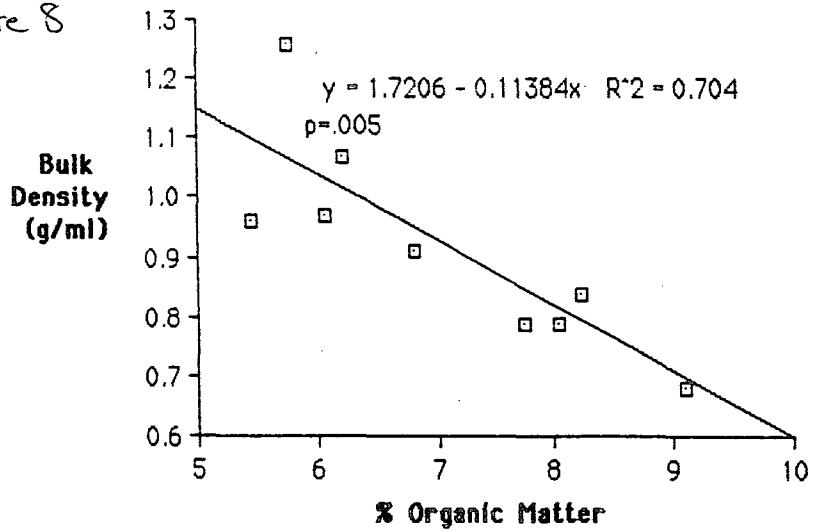
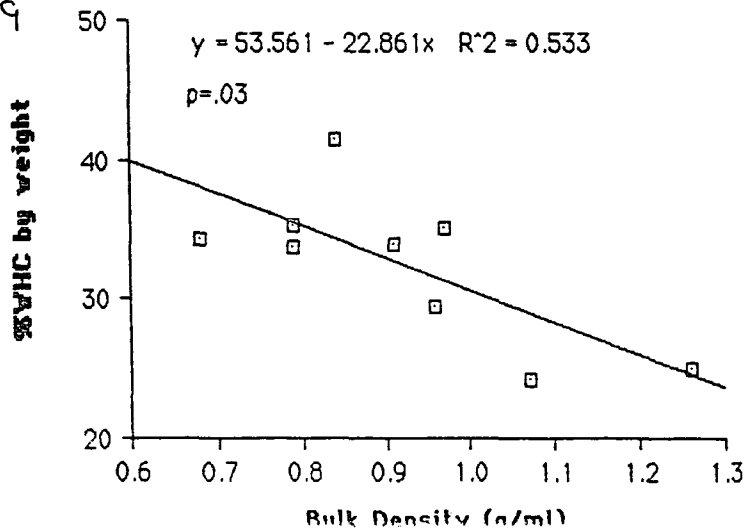


Figure 9



Appendix A

Regression Analysis of Associations Between Soil Properties

Figure 10

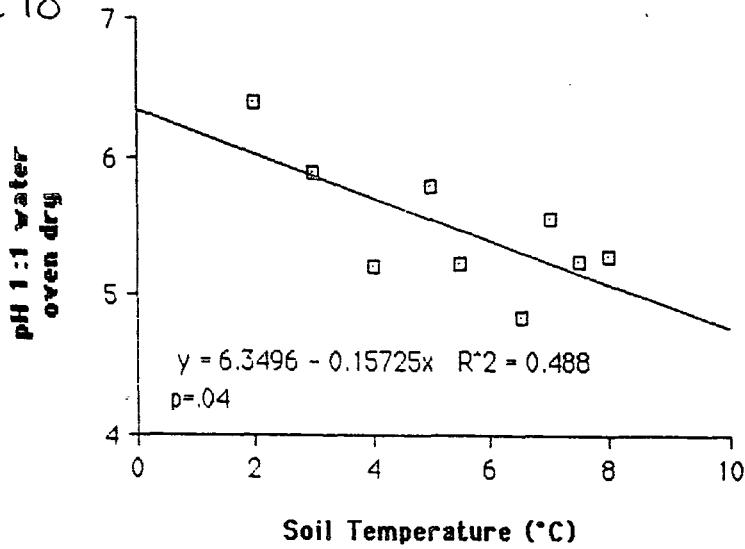


Figure 11

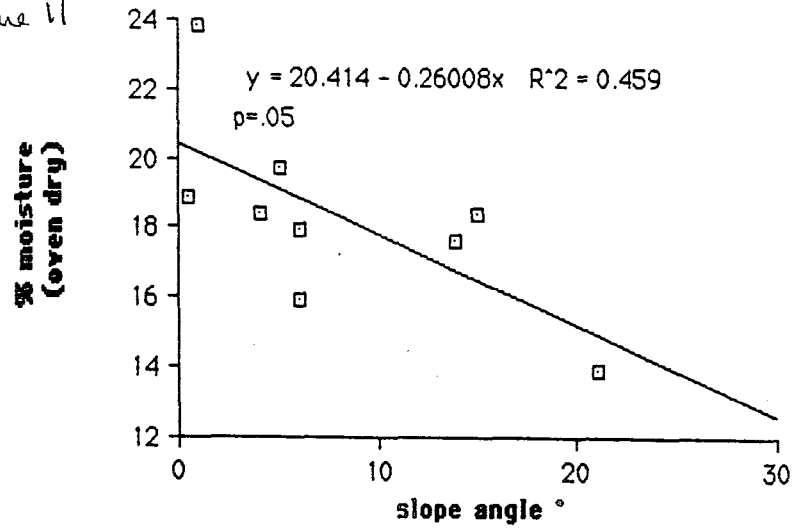
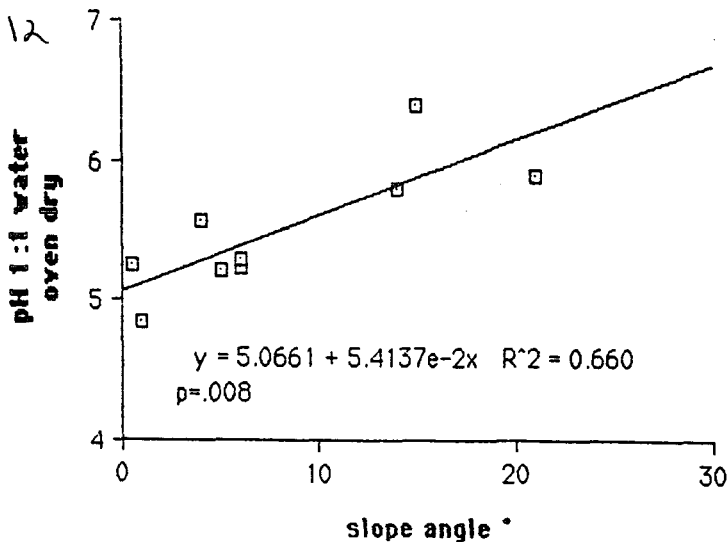


Figure 12



HISTORICAL BIOGEOGRAPHY OF *ASPLENIUM ADIANTUM-NIGRUM* (ASPLENIACEAE) IN NORTH AMERICA AND IMPLICATIONS FOR SPECIATION THEORY IN HOMOSPOROUS PTERIDOPHYTES¹

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Theories of plant speciation have generally recognized the importance of allopatry as a potential precursor to the genetic divergence of populations. The relative importance of long-distance dispersal vs. vicariance events in leading to allopatry, however, has been debated. We examined isozymic variability in highly disjunct populations of allotetraploid *Asplenium adiantum-nigrum* to test alternative hypotheses on their mode of origin. In addition, we assessed the genetic distinctness of the population from Boulder County, Colorado, which had been proposed as a separate species, *A. andrewsii*. Our results revealed that samples from all continental U.S. populations were isozymically identical and, with the exception of two samples from Boulder, displayed no intrapopulation variability. Continental U.S. populations were most similar to those from Hawaii, whereas both of these sets of populations were considerably more divergent from samples from Mexico and the Caucasus. The distribution of alleles and genotypes support the hypothesis that populations from different geographical regions had unique origins, resulting from at least several independent hybridization and polyploidization events followed by long-distance dispersal. These results have implications for speciation theory of pteridophytes in documenting the effectiveness of long-distance dispersal in the establishment of disjunct populations which may set the stage for allopatric speciation. In addition, the data suggest that the Boulder population is not sufficiently distinct to be considered a separate species.

Deciphering the historical factors that have produced present-day patterns of plant distribution can provide important insight into the dynamics of biogeographical processes and their interplay with mechanisms of speciation responsible for the origin and evolution of biological diversity. Although numerous detailed mechanisms of speciation have been identified, the general importance of allopatry is widely accepted (e.g., Coyne and Orr, 1989). The relative importance of long-distance dispersal vs. vicariance events in leading to geographical isolation and as a precursor to speciation, however, has been debated (e.g., Lynch, 1989). A difficult problem to overcome in this regard is that we are attempting to reconstruct historical events after the actual advent of speciation, where subsequent changes in geographic distribution could lead to erroneous conclusions on the original causes of geographical isolation.

Long-distance dispersal has been proposed as an important mechanism contributing to the widespread geographic distribution of many species of pteridophytes, the concomitant low rates of endemism generally found in

this group of organisms, and the apparent low rates of speciation compared to angiosperms (Tryon, 1970, 1972, 1986; Smith, 1972). Conversely, long-distance dispersal across particularly great distances could also allow for genetic divergence of newly formed populations in the absence of continued gene flow from source populations.

One avenue of investigation that may provide insight (albeit indirectly) into the relative importance of long-distance dispersal vs. vicarious isolation in speciation phenomena is the study of the origin of geographically disjunct, conspecific populations. Although we cannot know if particular disjunct, extant populations will ultimately diverge into separate species, numerous disjunct populations in the past undoubtedly have given rise to divergent evolutionary lineages. Thus, by discerning the mode of origin of disjunct populations, we may gain new knowledge into necessary preconditions for speciation events and of the relative impact of long-distance dispersal vs. vicariance in setting the stage for such precursors to speciation.

In the present study, we employed genetic data to explore the origins of highly disjunct populations of *Asplenium adiantum-nigrum* L. ("black spleenwort"). This is one of the rarest ferns in North America where populations are known only from Elden Mountain, Arizona, Boulder County, Colorado, and Zion National Park, Utah, and several locations in the Mexican states of Chihuahua and Tamaulipas. Outside of North America, *A. adiantum-nigrum* is known from the Hawaiian Islands, Europe, Africa, and Asia. Manton (1950) demonstrated that *A. adiantum-nigrum* is a tetraploid with $n = 72$ bivalents at meiosis I. Shivas (1969) provided cytological evidence² from experimental hybridizations suggesting that this tet-

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raploid is of hybrid origin, resulting from the hybridization of two European diploids, *A. onopteris* L. and *A. cuneifolium* Viv. The original diploid hybrid is presumed to have been sterile, but through chromosomal duplication (i.e., such as by nondisjunction at meiosis) ultimately produced a fertile allotetraploid. Existing evidence suggests that this is the most common mode of production of allopolyploid ferns, referred to as Type II polyploidization by Harlan and deWet (1975; see also Manton, 1950; Walker, 1979).

Ranker, Floyd, and Trapp (in press) suggested from genetic data that populations of *A. adiantum-nigrum* in Hawaii have resulted from at least several long-distance dispersal and colonization events from non-Hawaiian source populations. In the present study we address the question of the origin of the disjunct North American populations of this species. These populations could have arisen via long-distance spore dispersal from Old World or Pacific sources (Ewan, 1945) or they may be relictual from a once more continuously ranging species.

In addition to exploring alternative hypotheses on the mode of origin of the North American populations of *A. adiantum-nigrum*, we addressed the question of the genetic distinctness of the Boulder population which has occasionally been treated as a separate species *A. andrewsii* (Nelson, 1904).

MATERIALS AND METHODS

Because of the rarity of *A. adiantum-nigrum* in North America, minimal samples were taken to lessen the impact of collecting on the few existing populations. Leaves were collected from 21 individuals from each of the populations at Zion National Park, Utah (vicinity of Canyon Overlook Trail and Emerald Pools) and Elden Mountain, Arizona (bordering the city of Flagstaff). Twenty individuals were sampled from the population in Boulder County, Colorado (White Rocks area). Leaves were kept on ice in plastic bags until processing for electrophoresis. Specimens from the sampled populations are housed in the herbaria of Colorado State University (CS), New York Botanical Garden (NY), Northern Arizona University (ASC), U.S. National Herbarium (US), University of Arizona (ARIZ), University of Colorado (COLO), University of Wyoming (RM), and University of Utah (UT); new voucher specimens were not collected for the present study. Fragments of fertile leaves obtained from a population in the State of Chihuahua, Mexico (Mpio. Bocoyna, SE of Creel; Bye #18331) were kindly provided by Dr. Robert A. Bye, Jr. Spores were sown in petri dishes on an agar growth medium following Windham, Wolf, and Ranker (1986) to obtain gametophytes for isozymic analyses. Viable spores were also obtained from herbarium specimens collected in Mexico in 1977 (Bye 7384 from Mpio. Guachochi, Chihuahua; COLO accession no. 352634) and in the Sochi District of the Caucasus in 1981 (Vasák s.n.; COLO accession no. 441033) and sown on agar growth medium. The specimen from the Caucasus was chosen as a source of spores because its recent date of collection suggested that its spores would still be viable (i.e., see Windham, Wolf, and Ranker, 1986). Although the small samples available from Mexico and the Caucasus did not allow for estimation of intrapopulational variability, they were

sufficient to provide estimates of genetic similarity among populations (see Nei, 1978).

Samples were also collected from nine localities on the island of Hawaii, State of Hawaii. Detailed results from isozymic analyses of these populations are presented elsewhere (Ranker, Floyd, and Trapp, in press) and only general comparisons with Hawaiian populations will be made in the present context.

Horizontal starch-gel electrophoresis was conducted on extracts of collected leaf material and cultured gametophytes following Ranker et al. (1989). Results were obtained from 11 enzyme systems including aldolase (ALD), fructose-bisphosphatase (FBP), glutamate oxaloacetate transaminase (GOT), hexokinase (HK), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucose isomerase (PGI), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI).

Nei's unbiased genetic identity coefficient (I ; Nei, 1978) and Rogers' genetic similarity coefficient (S ; Rogers, 1972) were calculated for all pairs of populations with the program BIOSYS-1 (Swofford and Selander, 1989).

RESULTS

Seventeen putative duplicate locus pairs were scored across the 11 enzyme systems. Each locus pair was given a numeric abbreviation with the most anodal region of activity (when there was more than one such region on a gel) designated "1" (e.g., *Pgi-1*). The members of a pair were abbreviated with letters, "a" assigned to the more anodal region of activity and "b" assigned to the more cathodal region when two such regions could be distinguished (e.g., *Pgi-1a* and *Pgi-1b*; following Werth, 1989). Each locus of a pair was presumed to have been contributed by a single diploid progenitor of this allotetraploid species and, thus, the members of a duplicate locus pair were presumed to be homoeologous to each other (Klekowski, 1976; Werth and Windham, 1991). Because of the tetraploid nature of *A. adiantum-nigrum*, a region of activity that was expressed as a monomorphic, homozygous "locus" was interpreted as two monomorphic, homoeologous loci that were homozygous for the same allele. Allele frequencies for all populations are presented in Table 1.

The samples from Elden and Zion exhibited no within- or between-population variability and were fixed for a single allele at each of eight locus-pairs (*Fbp-1a/b*, *Hk-a/b*, *Idh-a/b*, *Mdh-2a/b*, *6Pgdh-1a/b*, *6Pgdh-2a/b*, *Pgi-1a/b*, and *Skdh-a/b*). At the remaining nine locus-pairs, all samples from Elden and Zion expressed multiple-banded patterns that were interpreted as fixed interlocus heterozygosity for functional alleles. Presumably these latter locus-pairs represent cases where the diploid parental taxa of *A. adiantum-nigrum* possessed different alleles that were combined in the allotetraploids (e.g., *Ald-a* contributed by one parent and *Ald-b* by the other parent). When assayed electrophoretically, individual gametophytes also expressed fixed heterozygosity at the same nine loci as the sampled populations (unpublished data). Values of Nei's I and Rogers' S were both 1.000 between the Elden and Zion populations (Table 2).

TABLE 1. Continued.

Locus	Population					
	Boulder	Zion	Elden	Mexico	Caucasus	Hawaii ^a
<i>Skdh-b</i>						
(N)	20	21	21	2	1	236
1	1.000	1.000	1.000	—	1.000	0.971
2	—	—	—	—	—	0.029*
3	—	—	—	1.000*	—	—
<i>Tpi-1a</i>						
(N)	20	21	21	2	1	236
1	—	—	—	—	1.000*	—
2	1.000	1.000	1.000	1.000	—	1.000
<i>Tpi-1b</i>						
(N)	20	21	21	2	1	236
3	—	—	—	—	1.000*	—
4	1.000	1.000	1.000	1.000	—	1.000
<i>Tpi-2a</i>						
(N)	20	21	21	2	1	236
1	—	—	—	—	—	0.005*
2	1.000	1.000	1.000	1.000	—	0.995
3	—	—	—	—	1.000*	—
<i>Tpi-2b</i>						
(N)	20	21	21	2	1	236
3	—	—	—	—	1.000*	—
4	—	—	—	—	—	0.110*
5	1.000	1.000	1.000	1.000	—	0.890

^a Values for Hawaii represent means of nine populations.

^b N = sample size per population per locus.

* Alleles unique to a region.

The Boulder sample was nearly identical to those from Elden and Zion, expressing a difference at only a single locus-pair. Whereas all samples from Elden and Zion and 18 of the individuals from Boulder expressed a "heterozygotic" genotype for *Lap-a/b* (combining *Lap-a*¹¹ and *Lap-b*⁴⁴, where superscripts refer to presumed genotypes), two individuals from the latter population were "homozygous" for allele 1 at this locus-pair (i.e., *Lap-a*¹¹; *Lap-b*¹¹). The only allele unique to continental U.S. populations, therefore, was allele 1 at *Lap-b*, found only in the Boulder population (but see discussion below on gene silencing). Rogers' *S* between Boulder and each of Elden and Zion was 0.997 (Table 2); *S* was a more sensitive indicator of the difference at *Lap-a/b* among these populations than Nei's *I*, which was 1.000 for both comparisons.

The samples from Hawaii exhibited more allozymic variability than the continental U.S. populations but generally showed high degrees of genetic similarity to them. Samples from Hawaii were invariant at eight locus-pairs (Table 1), four of which showed fixed "homozygosity" and four of which showed fixed "heterozygosity." The remaining loci exhibited varying degrees of variability within and among populations. Two of the heterozygous locus-pairs expressed three alleles and one expressed four alleles across individuals and populations, whereas only two alleles per locus were expressed at heterozygous locus-pairs in the continental U.S. populations. Twelve alleles unique to Hawaiian populations (so-called "private alleles") were observed across nine locus-pairs (Table 1).

Three of those private alleles (i.e., at *Hk-a/b*, *Skdh-a/b*, and *Tpi-2a/b*) were each found at only a single locality among the nine populations sampled on the island of Hawaii and were at frequencies of less than or equal to 0.110 across the entire Hawaiian sample. The remaining nine private alleles were discovered at two or more local populations and ranged in frequency from 0.004 to 0.721. The two private alleles observed at *Idh-a/b* (alleles 1 and 3) were found in all Hawaiian populations sampled. The mean values of *I* and *S* (Table 2) between Hawaiian and continental U.S. populations were 0.930 and 0.903, respectively. At three locus-pairs (*Hk-a/b*, *Pgi-1a/b*, and *Pgi-2a/b*), alleles were shared uniquely between Hawaiian and continental U.S. populations. Allele 3 at both *Hk-a* and *Hk-b* was fixed in all continental U.S. samples and was nearly fixed in all Hawaiian populations. At *Pgi-1a/b* all samples from both the continental United States and Hawaii were fixed for the same alleles. At *Pgi-2a/b* all samples from the continental United States and most of those from Hawaii were fixed for alleles 2 and 4, which were shared uniquely between these two regions; a subset of individuals from three Hawaiian populations were "homozygotes" for allele 2.

The samples from Mexico and the Caucasus showed the greatest amount of genetic divergence compared to each other and relative to all other populations sampled. Nine private alleles were found in the Mexican samples across six locus-pairs, and ten such alleles were discovered in the Caucasian sample at six locus-pairs (Table 1). Although the two Mexican samples were collected from different localities in Chihuahua, they were identical to each other at every locus. Mean values of *I* and *S* between the Mexican samples and those from the continental United States were 0.647 and 0.646, respectively (Table 2), and those between the Caucasus and continental United States were both 0.618. The mean values of *I* and *S* between Hawaiian and Mexican populations were 0.566 and 0.553, respectively, and those between Hawaiian and Caucasian populations were 0.577 and 0.573, respectively. The values of *I* and *S* between Mexican and Caucasian populations were both 0.529. Seven alleles were shared uniquely among Hawaiian and all North American populations, six were found only in samples from the Caucasus, Hawaii, and the continental United States, and four alleles were shared uniquely between the Mexican and Caucasian samples.

DISCUSSION

The nearly complete genetic identity among the three continental U.S. populations of *A. adiantum-nigrum* (Boulder, Elden, and Zion) suggests that they share a common and recent evolutionary history. All of the individuals sampled from Elden and Zion and most of those from Boulder may be descendants of a single hybrid individual that combined the particular parental genotypes expressed in those populations. The production of such an individual in one locality with subsequent spore production and dispersal to the present population sites would account for the observed distribution of this genotype in North America. (Ranker, Floyd, and Trapp, in press, present evidence suggesting that *A. adiantum-nigrum* is highly

TABLE 2. Matrix of Nei's unbiased genetic identity (above diagonal) and Roger's genetic similarity (below diagonal) coefficients between pairs of populations.

Population	Boulder	Zion	Elden	Mexico	Caucasus	Hawaii
Boulder	—	1.000	1.000	0.646	0.619	0.930
Zion	0.997	—	1.000	0.647	0.618	0.930
Elden	0.997	1.000	—	0.647	0.618	0.930
Mexico	0.644	0.647	0.647	—	0.529	0.566
Caucasus	0.619	0.618	0.618	0.529	—	0.577
Hawaii	0.902	0.903	0.903	0.553	0.573	0.994 ^a

^a Mean Nei's unbiased genetic identity among Hawaiian populations.

inbreeding, if not totally selfing, and thus does not experience sexual recombination. The equivalence of the number of genotypes with the number of hybrid origins, therefore, appears to be a valid assumption in this species.) Alternatively, the existing populations could be remnants of a once more continuous, southern Rocky Mountain metapopulation, most of which has gone extinct. Even under the latter scenario, however, one must invoke dispersal from a single original point of hybridization with subsequent reduction in population size.

The genetic identities among the continental U.S. populations may be particularly significant in unraveling the biogeographic history of this species in light of its tetraploid nature. Werth and Windham (1991) proposed a model of speciation of allopatric populations of polyploid pteridophytes involving reciprocal silencing of alternate alleles at homoeologous loci, and several empirical studies have documented such reciprocal silencing in disjunct, conspecific populations of allopolyploids (e.g., Werth, Guttman, and Eshbaugh, 1985b; Bryan and Soltis, 1987). No differential gene silencing has occurred between the Elden and Zion populations of *A. adiantum-nigrum*, however, supporting the recent common evolutionary history of these populations. The variant *Lap-b* genotype observed in two of the samples from the Boulder population may have arisen through the mutational silencing of allele 4 at this locus in an individual tetraploid plant or via a second hybridization event with both of the hybridizing individuals of the parental species contributing allele 1, and subsequent dispersal (directly or indirectly) to the Boulder locality. The gene silencing hypothesis seems more likely, given the otherwise complete genetic identity of the two variant samples from Boulder with all other Boulder, Elden, and Zion samples (i.e., across all other locus-pairs), in contrast to the great genetic differentiation observed among populations from different regions.

The fact that the most common genotypes in individuals from Hawaii were shared with those sampled from the continental United States suggests a common geographical and evolutionary origin for the populations in these two regions. This hypothesis is strongly supported by the presence of high frequency alleles (i.e., at *Hk-a/b* and *Pgi-2a/b*) that are shared between these two regions but not with the Caucasian population. Since the Hawaiian Islands arose through suboceanic volcanism in the mid-Pacific, we know that *A. adiantum-nigrum* is capable of successful long-distance migration to and establishment on previously uninhabited sites (Ranker, Floyd, and Trapp, in press). Whether the continental U.S. populations served as a source of migrants for the colonization of Hawaii (or

vice versa) or both regions received migrants from a third common source cannot be completely resolved from these data. Given that continental U.S. populations contain only a subset of the genetic variability present in Hawaiian populations, however, it is more likely that migrants from Hawaii were the source of colonists to North America rather than the other way around, if dispersal has occurred from one region to the other.

The extreme genetic differences among populations from the continental United States and Hawaii vs. those from Mexico and the Caucasus suggest that each set of populations originated independently of the others. The presence of unique alleles and genotypes at single locus-pairs and across locus-pairs in each region (combining Hawaii and continental United States) provides evidence that each set of populations arose from separate hybridization events between individuals of the parental species of different genetic constitution. The present European distribution of the putative parental species (*A. onopteris* and *A. cuneifolium*) suggests that hybrid individuals originally arose in Europe with subsequent dispersal events to Asia, Hawaii, and North America (either directly or via a "stepping-stone" sequence). Within North America, the unique alleles and genotypes distinguishing the populations from the United States and those from Mexico are best accounted for by hypothesizing separate hybridization events combining different parental genotypes with subsequent independent dispersals to the two regions. This hypothesis is most strongly supported by the distribution of genotypes between the two regions at those locus pairs that expressed completely different genotypes, i.e., *Hk-a/b*, *Lap-a/b*, *Mdh-1a/b*, *Mdh-2a/b*, *Pgi-1a/b*, *Pgi-2a/b*, and *Skdh-a/b*. Alternatively, the parental diploids could have occurred in earlier times in the continental regions where the tetraploids presently occur, with hybrids being produced in situ and subsequent extinction of the diploids from all regions but Europe. The nearly complete lack of genetic variability in continental U.S. populations combined with the presence of variability among populations from different geographic regions (thus indicating the polymorphic nature of the ancestral diploids) argues against the alternative hypothesis of multiple in situ hybridizations at the present North American localities. Although the alternative hypothesis of relictualism from ancient populations cannot be completely rejected, under that hypothesis one might expect more genetic variability within populations of *A. adiantum-nigrum*.

The recognition of the Boulder population as a species distinct from other populations of *A. adiantum-nigrum* (as *A. andrewsii*; Nelson, 1904) appears to be unwarranted in light of the present genetic data. It is worth noting, however, that the only genetic variability discovered among continental U.S. populations was found in the Boulder population. The unique genetic attributes of some members of this population are significant in terms of the conservation management of this rare species in the United States. Similarly, the individuals sampled from Mexico are genetically unique.

The results of this case study have implications for speciation theory in pteridophytes in favoring the hypothesis that the process of long-distance dispersal has led to the origin of the disjunct North American populations of *A. adiantum-nigrum*. The geographic isolation

of these populations from each other and from Old World and Pacific conspecific populations may allow for their ultimate genetic divergence leading to the origin of distinct phylogenetic lineages. The maximum levels of genetic divergence observed among populations in the present study are similar to those observed between the diploid species *A. montanum* and *A. rhizophyllum* of the Appalachian *Asplenium* complex (Werth, Guttman, and Eshbaugh, 1985a). Each of the genetically and geographically distinct entities within *A. adiantum-nigrum* in North America and elsewhere may experience different evolutionary fates, and because of the potential for the development of reproductive isolation (i.e., through differential gene silencing, other mutational events, and natural selection), these may represent cases of incipient speciation.

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