Experiment to Compare Stomata Density in Different Dicotyledonous

Aim: To investigate if stomata density on leaves in different dicotyledonous plants is effected by there country/ eco-system of origin. I will also compare the upper and lower epidermis stomata density to see were it lies.

Information on stomata and Hypothesis Based Upon this Information:

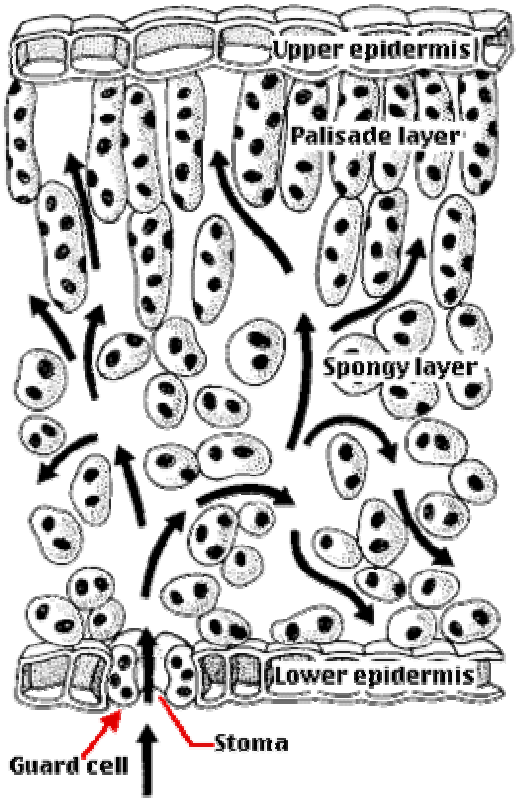
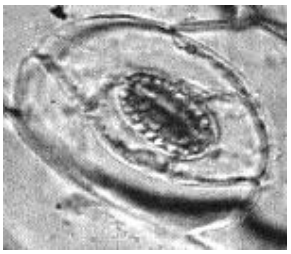


Diagram 1: Structure of a leaf.

The lower and upper epidermis along with the stem of a plant may contain stomata. These are openings through which gases are exchanged with the atmosphere and water is lost, this is called transpiration. Carbon dioxide is need in the process of photosynthesis. Carbon dioxide is diffused in through the stomata for photosynthesis and some carbon dioxide is produced through respiration along with the production of water which transpires out. These openings are surrounded by specialized crescent shaped guard cells, which changes their size and shape to change the size of the stomatal openings. This regulates the gas exchange e.g. open more gas exchange, closed no gas exchange. These guard cells have different stimulus to active or deactivate the openings; light, CO2 concentration, humidity and wind speed. The epidermis is covered with a waxy coating called the cuticle, which functions as a waterproofing layer and which helps to reduces water loss from the plant surface through evaporation. Transpiration (evaporation of water from plant surfaces) happens mostly from the surface openings, the stomata. Stomata transpiration accounts for most of the water loss by a plant, but some direct evaporation also takes place through the surfaces of the epidermal cells of the leaves.



Micrograph Pictures of Stomata

The Leaf is the principal food-making part of a plant. Not all leaves are green; many have additional pigments that produce colours other than green. The shapes and structure of leaves are adapted to the conditions which they live in.

Stomata represent a hazard to a plant in that they may cause excessive transpiration of water from the leaf. So if a stomata opens a leaf may lose excessive water, particularly if it lives in a dry, hot or windy habitat. Also, if it closes them it may run short of carbon dioxide or oxygen and also limit active uptake of water from the ground. Plants can resolve these problem by reacting to external stimuli and thus not opening them for longer than is necessary or vice versa (see guard cells for more information on stomata opening and stimuli). Stomata density can also be controlled by plant to limit risk factor of excess water loss. This however does not mean that a plant never loses more water than it can replace from the soil. The basic observation is that plants, not adapted to hot, dry, humid conditions, wilt in hot weather as they lose excessive water (when the stem of a plant lose it's turgid ness and thus mechanical support of the plant). Plants which live in climates with a moderate humidity (e.g. Britain) are different from those of humid, tropical regions or cold, dry regions. Most leaves have flat blades that expose as much surface as possible to sunlight as to produce as much food as possible, but a plants have adapted their leaves to their environment through evolution. Leaf adaptations:

★ Conifers: are adapted to cold, windy regions which they are native to. They have needle-like leaves that offer a minimum surface to the drying, winter winds. The leaves have one or two veins deeply imbedded in the middle and a layer of strong supporting tissue just beneath the thick and heavily cutinized outer layer.

★ Succulents and cacti: are adapted to arid regions. The leaves are often much more spongy and can retain large amounts of water. They often have a waxy cuticle on both the upper and lower epidermis to minimize water loss. The number of leaves is often reduced to minimize water loss. Stomata opening often only occurs at night to try an minimize further water loss.

★ Tropical forest plants: are adapted to allow excess moisture to run off at the tips, by different shapes and sizes of leaves. The high humidity means low rate of transpiration.

The stomata density thus can also be varied by a plant to suit it's conditions accompanied by other specializations. Density of stomata on a leaf therefore may be varied by temperature, humidity, light intensity and CO2 concentration.

For this investigation I choose to use dicotyledonous plants instead of monocotyledon plants. I could not use mixture of plants because monocotyledons plants have rows of stomata and are evenly distributed, whilst dicotyledonous plants have a randomised spread of stomata density. Monocotyledons plants, in general have long point leaves such as iris, maize and wheat plants. They produce a 1 seed leaf and have a number of protoxylem groups in the root. Dicotyledonous plants are 2 seed leaves such as buttercup, sunflower and lime tree. They usually have broad leaves with a vein network. The have s small number of protoxylem groups in the root. Comparing the two types of plant would have caused inaccurate results due to their many differences, making it impossible to compare the two types correctly or with any real judgement.

Guard Cells: Each guard cell contains chloroplasts, unlike the adjacent epidermal cells. The concentration of glucose in the cells changes with the photosynthetic activity and therefore it is the guard cells that regulate the opening and closing of the stoma.

Normally stomata open when the light strikes the leaf in the morning and close during the night. Also, when there is an increase in the osmotic potential, there is a decrease in the water concentration and therefore water moves into the guard cells by osmosis in response to the concentration gradient. As a result the thin-cell wall bulges into the epidermal cell pulling the thick wall with it and therefore opening the pore, making the cells turgid. Thus, when the guard cells become less turgid (loss of water) the pore subsequently closes. The alteration in the size of the stomata occur in response to a variety of the external stimuli such as light, carbon dioxide concentration and water.

The increase in osmotic pressure in the guard cells is caused by an uptake of potassium ions (K^+). The concentration of K^+ in open guard cells far exceeds that in the surrounding cells. This is how it accumulates:

1. Blue light is absorbed by phototropin which activates
2. a proton pump (an H^+-ATPase) in the plasma membrane of the guard cell.
3. ATP, generated by the light reactions of photosynthesis, drives the pump.
4. As protons (H^+) are pumped out of the cell, its interior becomes increasingly negative.

This attracts additional potassium ions into the cell, raising its osmotic pressure.

From a previous experiment, also counting the stomata in the upper and lower surface area to gather information of where the stomata density lies. The results below had be gathered. This experiment was undertaken in using a 10 X 8 magnification.

Leaf Type		No. of Upper Surface Stomata	Av.	No. of Lower Surface Stomata	Av.	Leaf surface type
Holly	1	0	9.33	200	206.33	Waxy cuticle, a very strongly
	2	13		220		structured leaf. Very rigid.
	3	15		196		
Laurel	1	0	2	200	199.66	Waxy upper surface area
	2	2		200		
	3	2		199		
Succulent	1	29	32	30	31	Both surfaces had a thin
	2	31		28		waxy cuticle, a juicy leaf
	3	36		35		
Choisya	1	3	2.67	148	147.66	Upper surface area had
	2	3		135		a waxy cuticle
	3	2		160		
Skimmia	1	13	13	480	440.66	Waxy cuticle top
	2	12		322		
	3	14		140		
Grape Ivy	1	1	0.67	73	76	Large leaf with thin waxy cuticle.
	2	0		75		
	3	1		80		

From this I worked out that the mean average of stomata on the lower surface area is 1101.31 6 = 183.55. However this is only a very approximant average as there is only a small range of plant results to take and there is mixture of high and low results to thus altering the average. However, in my prediction I will be able to relate my prediction of the stomata density average, giving me a base of reference.

Plants to be used and Predictions of Stomata Density for Each Plant:

Rhododendrons: About 700 species occur in the Himalayas of northeastern India, Burma, and southwestern China, and an additional 300 or so species occur in New Guinea. They exhibit great variety in size, habit, and flower colour, ranging from small, ground-hugging shrubs to small trees. India temperatures range from 14–31 degrees, with an average rainfall of 662mm up to 1935mm in some area.

- Due do there natural ecosystem; reasonably hot areas and the large amounts of rain they receive in the rainy season I would predict that there will be reasonably highly stomata density, and above the average.

Crassula Gollum: This plant originates from South Africa and is a succulent plant (holds water). This plant tends to grow low on the ground in rocky surroundings. They have thick, trumpet like leaves. Green with red/ purple edging. No visible veins, and the waxy cuticle covers the whole leaf. South Africa's temperatures range from 22 –11 and has an average rain fall of 751mm a year.

- As this plant is a succulent, adapted to hot, dry conditions I predict that there will be a low stomata density, in the plants aim to conserve water. The table above also shows a succulent with an average of 31 stomata on the lower epidermis, and thus I predict the stomata count will be below 50 for both the upper and lower epidermis. I predict this due to the fact that many succulents have a complete waxy cuticle covering that there will be approximately the same stomata density on both the upper and lower epidermis. The crassula gollum should also have a very similar stomata density compared to the crassula argenta as they are variants of the same species.

Money Plant (crassula argenta): Likes partial shade and originates from South Africa (see crassula gollum above for more climate info.). The minimum temperature that this plant can stand is 5 degrees. Lots of leaves on the plant and the leaves have no pronounced veins. The leaves are thick and spongy with a waxy cuticle on both the upper and lower epidermis.

- This plant is also a succulent, with thick spongy leaves, and thus I predict that the stomata density will be low, below 50 as for the crassula gollum. The whole leaf is also covered in a waxy cuticle to prevent water loss and thus, as predicted with the crassula gollum shall have approximately the same stomata density on both the upper and lower epidermis.

Christmas cacti: This plant originates from the South American jungles and is not a true cactus, and thus is not adapted so well to drought as may be expected. This plant enjoys partial sun and warm conditions.

- As this plants original ecosystem is the South American jungles the humidity is relatively high and good supply of water (rain) should be constant then the stomata density should be high, but to counteract this high

temperatures should lower the stomata density along with its cacti adaptations. Thus I predict that the stomata density will be below the average of most plants. Also this plant also has a complete waxy cuticle on both the upper and lower epidermis and thus will have approximately the same stomata density on both the lower and upper epidermis.

Evergreen Clematis: They are scattered over the temperate countries of the world. Many of the varieties are perennial vines, popular for covering fences and arbours. Each leaf has three pronounced veins and clearly visible network of veins. They have large leaves with a thin waxy cuticle on the upper epidermis.

- This plant lives in warm conditions, and is not adapted for extreme weather, thus I predict an average stomata of around density 180 stomata on the lower epidermis will be present.

Ivy: The plant's adventitious rootlets attach to trees or bare walls. The English ivy is commonly cultivated in Europe and North America in gardens, where it is trained to cover the walls of buildings. It has small leaves that are usually dark green.

- This plant is used to wet conditions and warm, and is not adapted for extreme weather, thus I predict and average stomata density (according to the above table of a previous investigation) will be present.

Camellia: A species of evergreen shrubs or trees of the tea family. They are native in tropical and subtropical Asia. Camellias are grown in warm, damp regions in various parts of the world. Camellias have quite thick leaves with a very thick waxy cuticle on the upper epidermis.

- Due to the very thick waxy cuticle I predict that there will be a very low stomata density on the upper epidermis. As they grow in damp, humid conditions this means that there will be a low transpiration rate and lots of active uptake of water meaning a high stomata density on the lower epidermis, above the average set by the previous investigation.

Holly and the Speckled Holly: The holly family consists of trees and shrubs usually having separate staminate and pistillate flowers that are small in size, four- to eight-parted in structure, and white or greenish in colour. The fruits are usually red drupes, containing two to eight one-seeded stones. English holly is a small tree with spiny evergreen leaves and bright-red fruit. Holly leaves in general are thin with ruffled edges. Pronounced main vein and smaller vein network. Thick waxy cuticle on surface.

- These two species are both well adapted to damp, warm weather and thus should have a average stomata density, as set by the previous investigation.

Geranium: This is a common plant found in Britain today, however it originates from Iran/Central Asia. It grows better in sunny, warm conditions but can also cope with frosty conditions outdoors. The leaves are quite large with small hairs covering the upper epidermis so lower transpiration rate as the hairs create diffusion cells of water around the stomata making an area of high humidity and thus lowering transpiration. There is no waxy cuticle present. The temperature in Iran ranges from 2 – 29 and the average rain fall in one year is 255mm.

- ★ The geranium is native to a quite hot dry climate, and although it can withstand cold temperatures it is still adapted to hot, dry weather i.e. the hairy leaf. Therefore I would predict a low stomata density on both the upper and lower epidermis.

Water Lily: The leaves float on the water's surface, maintaining their position, even if the water rises, by continuing stalk growth. Water lilies typically grow in deep, quiet waters. Oxygen needed for root growth is supplied by air passages extending down the leaf petioles. Two species were both represented in ancient Egyptian art. The family also contains the gigantic water lilies of the Amazon.

- ★ As this plant has a continues water supply means it has little need to conserve water, thus I would expect an extremely high stomata density to be shown.

Lily of the Valley: The cultivated species is native to temperate Eurasia and is probably of introduced origin in North America. Lily of the valley has long been a favorite for shady gardens, where it will form a dense stand to the exclusion of all other vegetation.

- ★ This plant likes shade and thus would suggest relatively high stomata density as the leaves are out of the sun. However, it's original ecosystem is quite hot and this would lower the stomata density, bringing down to around the average stomata density, as set by the previous investigation..

Eucalyptus: This plant is native to western Australian forests. Eucalyptus trees are characterized by leathery, whitish leaves that hang vertically, their edges facing the sun. The leaves are often a silvery colour to help deflect the strong sun rays. Australia temperatures range from 6 – 20 degrees in some areas and up to 25 – 28 degrees in other with an average range fall of 585mm in some areas up to 1502 in others areas.

- ★ As this plant is native to very hot, sunny condition and relatively low rainfall in some areas and with a complete waxy cuticle I would predict a low stomata density. However, one adaptation of the eucalyptus is the positioning of their leaves so as not to be indirect sunlight, which may increase the stomata density.

Bramble: The ripe fruit is an aggregate of small, purplish-black drupes attached to a cone-shaped receptacle, which readily separates from the plant when the berries are picked. It is thought the common Bramble (Blackberries) originates from Eurasia, central Asia. The whole of the upper epidermis is covered with large hairs and on the lower epidermis there is a pronounced vein network.

- ★ The small hairs would reduce transpiration rate and thus may increase stomata density. However, this plant is native to a reasonably hot region and thus I would predict an average stomata density, as set by the previous investigation..

Saintpaulia (African Violet): All the different variants of this species are native to the mountains of Tanzania and Kenya in East Africa. The leaves are thick and spongy, and both sides are covered in hairs and pronounced vein network. The temperature in Kenya and Tanzania ranges from 24 – 27 degrees.

- ★ This plant is native to a constantly hot, dry and relatively windy environment and the thick spongy leaves and hair all reflect his environment by minimizing water loss. Thus I predict a very low stomata density compared to an mean average stomata density, as set by the previous investigation..

Fatsia Japonica: A plant with very large deeply lobed (7-11 lobes) glossy green leaves up 16 inches wide. This plant has a waxy upper epidermis cuticle. Geographic Origin is South Korea, Japan. This plant enjoys full sun to light, dappled shade and moist but well drained soil. The temperature in South Korea ranges from 5–24 degrees whilst the average rain fall in one year is 1425mm.

- ★ The temperature and high average rainfall of this plant country of origin suggest that the plant does not need drastic water retention devices and thus I predict that the stomata density will be relatively high.

Laurel: This is common name for a flowering plant family, widespread in tropical and subtropical regions. The laurel family contains between 30 and 50 genera and at least 2,000 species. The leaves are thin with a thin waxy cuticle. The leaves are also quite hard and inflexible.

- ★ Living in subtropical or tropical region would mean relatively high temperatures, but high humidity and good water supply. This would all indicate little need to conserve water. This is why I predict that the stomata density will be high for the Laurel.

Honeysuckle: It grows mainly in the North Temperate Zone, particularly in China, but it also extends into tropical mountains. Its members are mostly shrubs or small trees. The leaves are opposite one another on the branch. The leaves have little, if no waxy cuticle and are very soft and fragile.

- ★ The lack of waxy cuticle may mean this plant needs to conserve the plant in different ways i.e. a lower stomata density.

Primrose: This plant grows in North Temperate Zones of the earth. In Britain the plant grow in the spring covering forest floors. Long leaves with highly textured surface. No waxy cuticle present. Spongy leaf.

- ★ The plant is used to growing and flowering in the damp, coldish spring and thus would not need to conserve water so much and thus a relatively high stomata density would be predicted. However, this plant does not appear to have any waxy cuticle and evolution may have meant a reduction in stomata density to counteract this.

Dock (plant): Widely distributed in temperate areas of the world and grow in damp conditions. Dock plants usually have stout taproots and large basal leaves with little, if no waxy cuticle.

- ★ The dock leaf has no waxy cuticle immediately lowering the stomata density that I would predict. However, they enjoy damp conditions and

thus I predict an average stomata density, as set by the previous investigation.

Summary: I predict that plants from either hot, dry or cold, windy eco-systems will have a lower stomata density compared to plants living in low humidity, damp eco-systems. I also predict that the stomata density will decrease in the upper epidermis due to the waxy cuticle and the increased water loss due to sun exposure (and thus increased heat exposure), through transpiration here.

After looking at different plants you would expect most of then to be well adapted to there habitat in which the live, most of the plants are from hot and dry places like Asia and Africa where for a plant it is essential to prevent water loss as water is scarce in these kinds of places and the loss of excessive water could lead to problems for the plant.

Equipment:

1. Chosen plant leaves: variety of leaves to take my epidermis impressions from. In the preliminary investigation I will take impressions of both the upper and lower epidermis to conclude which epidermis has the highest stomata density. If, as I predict the lower epidermis on all/ most of the chosen plants does have a larger stomata density then I will only do a lower epidermis in the final experiment.
2. Clear nail varnish: to paint on to the leave to take an epidermis impression
3. Thin ended tweezers: to peel the epidermis impression off from the leaf
4. Glass slides. To place the impression onto and so I can look at any stomata present under a microscope.
5. Microscope: to observe and count any stomata present in the impression, as stomata are not visible to the naked eye
6. Tally counter: So that I count the number of stomata easily and so I can record my results easily, without relaying on human memory.

Risk Factor: There is very little danger, if any in this experiment. The risk factors are:

1. Clear nail varnish fumes are toxic to human. To counter this risk a well ventilated room shall be used to avoid inhalation of excess fumes.
2. Care in applying the varnish should also be taken to avoid spillages on to human skin, eyes or clothing. If the varnish comes in contact with eyes wash immediately at labelled eye wash stations and seek immediate medical attention.
3. If consumption of nail varnish occurs seek immediate medical attention
4. The pointed tweezers are sharp ended, thus care in carrying these should be taken to avoid accidents.

Preliminary Method:

1. Prepare an epidermal impression by coating the upper and lower leaf surface with a thinish coat of clear nail varnish.
2. Wait for approximately 15 minutes or until the vanish is completely dry. Then peel off the dried layer of nail varnish very carefully using a pair of thin ended tweezers. (Alternatively, with some plants you can

peel off an epidermal strip directly, which can be mounted in water on a slide and place under the microscope)

3. Place the impression on to a glass slide a smooth out any bubbles.
4. Place another glass slide over the top of the impression to hold it secure.
5. Using a microscope at a set magnification (to be decided through preliminary experiment) count the stomata that you can see. Making sure you do not move the slide at any time. Record these results and then recount this area at least three times, and then move the slide on to another area and count the stomata there, recounting three times.
6. Do this for each the lower and upper epidermis and for a variety of plants making sure you record all results.
7. Then take an average from the results. This is to allow for human error.

Preliminary Investigation: findings and adjustments. The only thing that I changed in my method was the use of cover slips instead of using another glass slide to cover the epidermis impression. I found that through using a cover slip I got increased visibility and more defined stomata as they were thinner than the glass slides and cleaner too. I also developed a method to remove a large section of a epidermis peel from the leaf cleanly and with out taking parts of the leaf with it (see Final method for more details). As I did not change the method significantly I have decided to build upon the results I gained this experiment and compare them with further results.

From the preliminary investigation I also decided what magnification is to be used. A 15X eye piece and 8X magnification = 8 X 15 = 160. I decided this by using different magnifications on each epidermis peel and this magnification gave good visibility of the varying size of stomata from plant to plant, helping to make my results more accurate.

A trend became apparent in the preliminary results through the graph "Bar Chart to Compare the Lower and Upper Epidermis Average Number of Stomata per 4.91mm from the Preliminary Results." All of the samples, apart from samples 5 and 6, showed that the mean average of stomata per 4.19mm on he lower epidermis is dramatically higher than that on the upper epidermis. This proves my theory that the overall stomata density would be situated on the lower epidermis. Although I have conclude in this preliminary experiment that the stomata density of a leaf was on the lower epidermis some plants like plant 5 and 6 (crassula argenta and the Christmas cacti) due to there environment and adaptations do have almost identical stomata density on both the upper and lower epidermis. Thus, I have decided to continue to look at the upper and lower epidermis to make sure that this stomata density doesn't change with different plants.

Adjusted Equipment List:

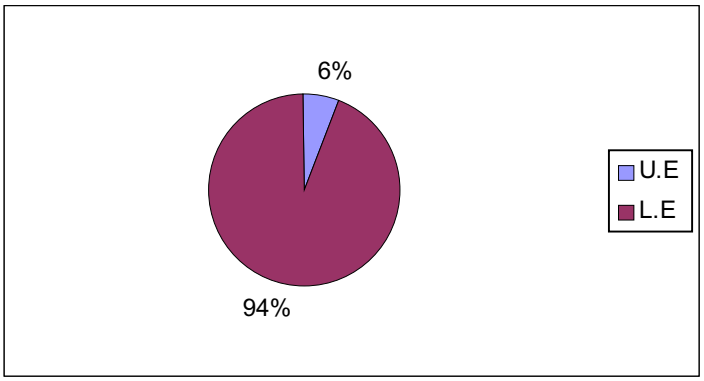
1. Dock, Primrose, Bramble, Honeysuckle, Eucalyptus, Fatsia, Saintpaulia, Holly, Speckled Holly, Rhododendron, Water Lily, Lily of the Valley, Laurel leaves: a wide variety of plants to be used to take both a upper and lower epidermis impressions from. in the preliminary experiment I did conclude that the stomata density of a leaf was on the lower epidermis I have decided to continue to look at the upper and lower epidermis to make sure that this stomata density doesn't change with different plants.

2. One pot of clear nail varnish: to paint on to the leave to take an epidermis impression
3. One pair of thin ended tweezers: to peel the epidermis impression off from the leaf
4. 13 Glass slides. To place the impression onto and so I can look at any stomata present under a microscope.
5. 13 Cover slips: to cover the epidermis impression once on the slide to flatten the peel and to make sure it is secure and will not be blown away or damaged.
6. One Microscope with a 15X magnification eye piece: to observe and count any stomata present in the impression, as stomata are not visible to the naked eye
7. One tally counter: So that I count the number of stomata easily and so I can record my results easily, without relaying on human memory.

Final Method:

1. Take your leaf of the chosen species and prepare an epidermal impression by coating the upper and lower leaf surface with a thinish coat of clear nail varnish.
2. Wait for approximately 15 minutes or until the vanish is completely dry. Then peel off the dried layer of nail varnish very carefully using a pair of thin ended tweezers. To do bend the leaf towards the end of the painted area. Hopefully the epidermis peel should start to separate from the leaf and the tweezers can be slide underneath and a large epidermis peel should come off easily.
3. Place the impression carefully on to a glass slide a smooth out any bubbles.
4. Place another a cover slip over the top of the impression to hold it secure and flat.
5. Using a microscope at the magnification of 8 X 15. Focus the microscope so that the stomata are clearly visible.
6. Count the stomata that you can see in this magnification. Making sure you do not move the slide at any time. Record these results and then recount this area three times. Then move the slide on to another area and count the stomata there, recounting three times.
7. Remove the slide form the microscope and place on the labelled chart in the correct position e.g. a lower epidermis peel of the primrose placed on the L.E. Primrose section for further reference or recounting.
8. Do this for each the lower and upper epidermis and for a variety of plants making sure you record all results. Then take an average of the six results for each species. This is to allow for human error.

Analysis: The result tables back up the trend presented in the preliminary results showing a obvious trend in the results, that the pie chart below illustrates, that the stomata density is clearly located on the lower epidermis.. The pie chart below shows that just 6% of the total stomata is located on the upper epidermis and 94% of the total stomata are located on the lower epidermis.



The pie chart is supported by both graph 1 and 2 (see foot note of the graph for numbering) both show that for all the plants that I managed to obtain epidermis impressions from, apart from 3, show that the stomata density lays on the lower epidermis. In all of the plants (apart form the 3 exceptions) show a massive difference in the number of stomata on the upper epidermis compared to the lower epidermis. The speckled holly showed a 100% increase of stomata from the upper to the lower epidermis, as there was an mean average of 97.60 stomata per 4.91mm on the lower epidermis to no stomata on the upper epidermis. Other plants, such as the lily of the valley showed an over 50% increase from the upper to the lower epidermis, going from a mean average of 34.17 to 75.50 stomata per 4.91mm. Although there are big differences in the number of stomata on the lower epidermis compared to the upper epidermis there appears to be no set pattern, or equation to the results. However, there were 3 exceptions to this rule, and they were the money plant (crassula argenta), crassula gollum and the Christmas cacti. As I predicted the number of stomata per 4.91mm was approximately the same on both the upper and lower epidermis, this is due to there environmental adaptations, a complete covering of a waxy cuticle, which means that both sides of their leaves are identical, and thus the stomata density is the same to.

From a previous investigation I had worked out an approximate mean average of stomata for the lower epidermis, so see if this average was approximately right I worked out a mean average for the lower epidermis using the mean average results for each plant from this investigation. This turned out to be: 2127.2/ 17 = 125.13 stomat a per 4.19mm, whilst the other average from the previous experiment was 183.55 this is quite a big difference of 58.42, the average of both of these is 183.55 + 125.13 / 2 = 154.31. However, the average would be different as I have used a different set of plants and a wider range of plants and I do not now how many stomata to what area it is for the mean average from the previous investigation. I will compare my to the over all average mean of the two combined results , from the previous investigation and from this investigation. This mean average of stomata for the lower epidermis covers a wider range of plants and would thus suggest a greater degree of accuracy.

My prediction for the money plant, Christmas cacti and crassula gollum were all proved correct. I predicted a stomata count of below 50 and an equal stomata density on both the upper and lower epidermis. Graph 1 shows all of these plants and almost equal level of the bars clearly show that the stomata density is almost the same on the lower and upper epidermis. Also all 6 mean average were way below 50. The money plant was 22.50 and 20.16 stomata per 4.91mm, the Christmas cacti was 16.60 and

20.10 stomata per 4.91mm and crassula gollum was 10.5 and 11.83 stomata per 4.91mm. These small deviations in the stomata density for the upper and lower can be put down to the random spread of stomata on the leaf surfaces. However, the Christmas cacti is meant to be adapted to the South American jungles, and although I predicted a lower that average stomata count (lower than all three averages I have worked out) I did not expect it to be this low, and this I can not explain. The Saintpaulia, also from South Africa has had the expect result of very low stomata count, just a mean average of 19.33 stomata per 4.91mm for the lower epidermis and 7.3 stomata per 4.91mm for the upper epidermis. These results for the Money Plant, Saintpaulia and Crassula Gollum prove that the country of origin and thus environment of origin do effect the stomata density for these three plants, as the plants have reduced there stomata density to adapt to the hot, dry climates of South Africa.

The rhododendrons, as I predicted had a very high stomata density of 210.50 stomata per 4.91mm, and this is 56.19 over the mean average. As for the South African plant this is evidence that the rhododendrons are adapted to their original environment.

I predicted for the evergreen clematis an average number of stomata, however the investigation showed that the results were way below that of the average at just 77.00 stomata per 4.91mm compared to the average of 154.31 stomata per 4.91mm. One explanation for this is that the plant has compensated more than I thought for the lack of a waxy cuticle, but I can not be sure. Another explanation is human error, and that counted the stomata wrong. However, this seems unlike as the results are fairly constant.

As I predicted for the ivy, the stomata count for the upper epidermis was near to average stomata count of 154.31 stomata per 4.91mm and the Ivy count was 170.00 stomata per 4.91mm. This helps to prove that ivy is adapted to its environment.

The camellia, as predicted, had a very high stomata count on the lower epidermis, with a mean average of 203.00 stomata per 4.91mm that's nearly 50 stomata over the total mean average. As graph 3 shows the camellia is a plant with one of the highest stomata density that I investigated. The results also help to prove that this plant has adapted to it's original environment.

I originally predicted that the two types of holly would have similar stomata count, however this was not shown in the investigation. The stomata count was 215.2ata per 4.91mm for the holly, and for the speckled holly it was just 97.60 stomata per 4.91mm on the lower epidermis. This is a big difference, and just proves that different variations of the same species have different adaptations for there environment e.g. leaf colouring, thickness of the waxy cuticle. He plain holly did prove my prediction right, being well over the overall average for the lower epidermis, but he speckled holly did not. One explanation for this is that this species has adapted it's self to another environment or has adapted a low stomata density due to other areas of the leaf not adapted to water retention.

The water lily, as I predicted had an extremely high stomata density on both the lower and upper epidermis. Graph 4 compares all the plants stomata count (mean average) as the chart shows the water lily has about 3 times the number of stomata on both the upper and lower epidermis than all of the other plants. This backs up my conclusion

that the water lily would have an extremely high stomata density due to it's watery environment. One key observation that I made during this investigation was that the stomata of the water lily appeared to have no guard cells, which were clearly visible on the other plants, instead just stomata, that looked like little black holes. This is further evidence that the water lily is highly adapted to it's watery environment, as it has no need to regulate it's transpiration rate as lack of water will never be a problem for the water lily.

The lily of valley results did not conform with my prediction. I predicted an average stomata density (of around 183.55) but the results showed a relatively low stomata density of 75.50 per 4.91mm for the lower epidermis and 34.17 per 4.91mm for the upper epidermis compared to revised overall average of 154.31. This is around 50% lower than the revised average and about 75% lower than my original expected mean average. The only explanation for this is that the climate is hot than I though and the plant has adapted to the heat through low stomata density rather than a thick waxy cuticle of reduced volume of leaves. Looking at either graph 2, 3 or graph 4 show that the stomata count is low compared to other species of plant (apart from the cacti and succulent species as well as the Saintpaulia).

In the eucalyptus I predict a relatively low stomata density, how ever the stomata count was close to eh overall mean average of 154.5 per 4.91mm, with a lower epidermis count of 122.60 per 4.91mm. Also by looking at graph 4 the eucalyptus is near the same level as many of the other plants mean average of stomata per 4.91mm on the lower epidermis. As I described in the prediction the eucalyptus does position its leaves so the edges face the sun. This adaptation and thick waxy cuticle could explain why the stomata density has not adapted so much as the succulent plants that I investigated.

The fatsia japonica was predicted as having a relatively high stomata density, however this was not shown in the results. As you can see on graph 4, the fatsia is near the average line, and is in fact below it an mean average of 166.00 stomata per 4.91mm. I have no explanation for this, as the plant needs damp soil, dappled shade and not very high temperatures.

For the laurel I predicted an above average stomata density, which it was with an average of 202.67, around 50 over the revised overall mean average. This shows that the laurel has adapted as I expected to it's tropical/ subtropical regions.

The primrose and dock mean average of stomata per 4.91mm was way below the overall average at with the primrose with just 57.60 stomata per 4.91mm. Like many of the other plants under the predicted stomata density these plants also had no waxy cuticle present, and could be adapting the stomata density to deal with lack of protection from water loss.

Summary: The graphs show me that the stomata density is located on the lower epidermis. My graphs have also helped me to explain and back up my predictions for each plant. Out of the 20 plants that I planned to investigate I got results for 17 of the, from these results only 9 of my predictions were proved correct. The predictions that were proved right were mainly for the plants that are adapted to extreme conditions e.g. the water lily. The other plants with moderate environments varied with stomata

density, either below or near the overall average of 154.5 stomata per 4.91mm. The only explanation for this the plant has other adaptations suited for it's environment, and thus the stomata density is not what you may expect e.g. lots of hairs to slow the transpiration rate or lack of a waxy cuticle.

Evaluation: Overall the experiment appeared to reasonably accurate, as from looking at the result tables there are very few anomalies in the stomata counting, although there were very few results were the stomata count was the same for each set of 3 counts, but these generally were only small differences. There are only 7 apparent anomalies out of 240 stomata counts. These anomalies occur with the first count of water lily and with the all the counts of the fatsia. From inspecting the table the water lily stomata count 1 (for square one) was 400 stomata, were as count 2 was 588 and count 3 was 540. This means count one was out by around 450 stomata. There is only one obvious explanation for this anomaly and this is human area. As I was counting the stomata I may miss some stomata or recount others as they are not in nice, even lines but randomly spread. In this cases I appear to have dramatically undercounted the stomata. There are 3 solutions for this 1) dividing the area of view in to 2 or 4 areas and counting the stomata in that area, so for a area divided in 2 I would then times the result by 2. However, as the stomata are spread out unevenly, with areas of high stomata density and large areas with no stomata this too is quite inaccurate. 2) To break the field of magnification in to small areas using a slide with prepared grid, thus counting each square methodically and thus reducing the chance of recounting stomata or losing my place of site 3) This is a very high-tech option, which would not be available to a school, but would make the results very accurate, and this is to use a electrograph to photograph the epidermis impression. This way I could then count and label/ cross off the stomata, making sure I had counted each one, or divide the picture in to grids and count them that way.

However, the anomaly's displayed for the Fatsia plant can not be explained so easily. Looking at the results of count 1, 2 and 3 for square one there is not too much difference between the results, however, for square two the results are all quite different ranging from 88 to 136 stomata per 4.91mm. As there is no real technical equipment being used in this investigation to count the stomata it may just be human error showing through again. On other possibility may have been the tally counter I was using, and may not have recorded each time I pushed the button. If this was the problem then I would in a following investigation have two tally counters and check often that they are working correctly. If not then I would swap to the other tally counter, and use that one instead.

One thing I have noticed form the result table is the margin of error increases as the number of stomata to be counted increase. For example the lower count for the upper epidermis is fairly constant for the laurel. The results for square one was: 1, 1, 2, then for square two: 3, 2, 2, meaning a range of only 1 for each set of results. These results are almost the same, but when counting the much large number of stomata on the lower epidermis the results for square one were: 148, 140, 142 and for square two: 140, 145, 149. The difference between each recount are much higher, for square on the range was 8 whilst for square two the range was 9. Thus, I have concluded that increased stomata density means increased human error. To counteract this human error I could do more recounts to gain a larger view of results and thus, it would be easier to spot abnormal results and I would also gain a more accurate mean average.

One of the biggest problems that I had to over come in this investigation was the correct removal of the nail polish (epidermis impression), or even a large enough piece to use from the leaf surface. There where 3 plants that I could not obtain any epidermis impression from and one plant, the eucalyptus, that I could not obtain a upper epidermis impression from. Also, some of the plants took more than coating of nail varnish to obtain an impression. The plants that I could obtain no epidermis impression from was the blackberry/ bramble, geranium and honeysuckle leaves. The bramble was like the geranium covered in millions of hairs that meant the nail varnish came off in little pieces that were too small to use, or the hairs came off in the nail varnish making the image under the microscope fuzzy and they covered many of the stomata. This would have made the results were inaccurate. Also, with the geranium and with he honeysuckle the leaves had no waxy cuticle to them. This meant that the nail varnish was absorbed into the leaf and did not dry, instead the leaf when dark green, soggy and very fragile, making it impossible to gain an epidermis impression like this. For the eucalyptus obtaining the lower epidermis impression was very difficult and when it came to the upper epidermis I just could not get a piece large enough. I found that, although the leaves did have some waxy cuticle , he nail polish just did not come off, and the leaf seemed to absorb it. Other plants that I had trouble with was the primrose, which like the geranium, soaked up the nail varnish. However, after several re-painting of the leaf I did manage to get an epidermis impression. To stop this from happening in the further investigations you could do an epidermis peel, this is were the epidermis is removed directly from the leaf and looked at under the microscope.