Variation in leaf attributes and their effects on physiological processes in *Leucospermum conocarpodendron* (L.) Buek.

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Abstract:

Two subspecies of *Leucospermum conocarpodendron* with considerable variation in leaf traits occur along the Cape Peninsula along clearly delimited geographical distributions. We attempted to quantify the difference in leaf trait dimensions between green (*L. conocarpodendron* ssp. *viridum*) and grey (*L. conocarpodendron* ssp. *conocarpodendron*) individuals. Leaves from grey individuals are shown to have higher reflectance across the entire photosynthetically active region (PAR) and greater stomatal density. Leaves from grey individuals were found to have higher specific leaf area (SLA) and a greater size boundary layer. We found no correlation between the transpiration rate and stomatal density, size of the boundary layer and hairiness. We hypothesize that the leaf traits are having an effect on rate of photosynthesis and subsequently determining growth strategy of each type. Grey individuals, because of increased reflectance from the leaves are able to persist throughout summer in hot, dry conditions, although they may suffer the cost of reduced photosynthetic rate during late winter and early summer when conditions are relatively mild. Green individuals are able to start growing earlier due to higher SLA and increased rate of photosynthesis but are not able to persist for as long as the grey individuals during mid to late summer. Finally we argue that adaptation to edaphic environment may be associated with subsequent shifts in flowering phenology.

Introduction:

Rourke (1972) reported that there is considerable variation in the form and dimensions of the leaves in *Leucospermum conocarpodendron*, a large shrub found in the Cape Floral Region of South Africa. Rourke (1972) recognized two subspecies based on the presence or absence of a crisped canescent indumentum on the leaves. *L. concocarpodendron* ssp. *conocarpodendron* has canescent leaves with a dense indumentum of fine crisped hairs, only becoming glabrescent after several years. *L. conocarpodendron ssp. viridum* has dark green, glabrous leaves. Individuals of the two ecotypes grown from seed have remained true to form after about four years which suggests that the presence or absence of a crisped indumentum is a genetically stabilized character (Rourke 1972). Rourke (1972) also reported considerable variation in leaf form and size (Fig. 1), although no attempt was made to determine whether this variation in leaf form and size was consistent with the subspecies delimitation.

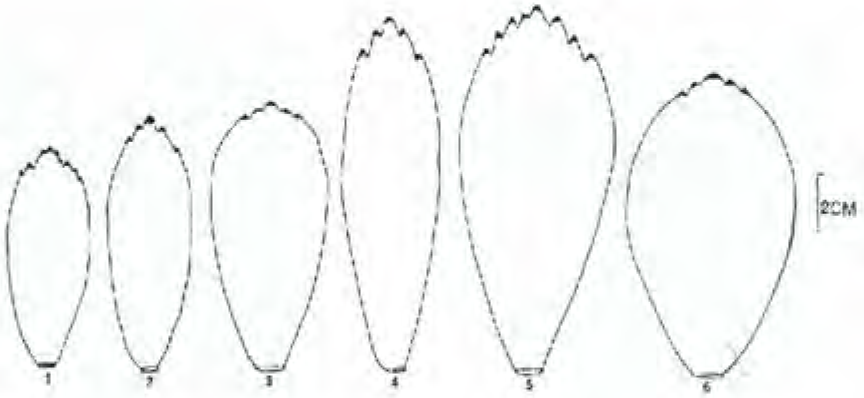


Fig. 1: Variation in the size and form of leaves of *Leucospermum conocarpodendron* (L.) Buek (from Rourke 1972).

Rourke (1972) reported that there is clear geographical delimitation of these two forms (Fig. 2). The form with dark green, glabrous leaves occurs as far north as Kirstenbosch, on the south-eastern slopes of Table Mountain but is separated from the form with canescent leaves by the evergreen forests at Kirstenbosch and Newlands (Fig. 2; Rourke 1972). The most southerly population of the form with canescent leaves is on Little

Lion's Head, above Llandudno and is almost contiguous with the glabrous form which occurs on the adjacent Karbonkelberg (Fig. 2; Rourke 1972). There are a number of populations of the grey (canescent) form on the eastern slopes of Devil's Peak, along the western and northern slopes of Table Mountain and along the Twelve Apostles up until Llandudno (Fig. 2). Rourke (1972) reported that a "hybrid swarm", containing every intermediate between the two forms, is present on the eastern slopes of Little Lion's Head. This project aimed to investigate the possible causes and consequences of these differences in leaves between the two subspecies.

Fig. 2: Distribution of *Leucospermum conocarpodendron* (L.) Bueck ssp. *conocarpodendron* (open circles) and ssp. *viridum* Rourke (closed circles).

A number of studies have investigated the adaptive significance of variation in vegetative properties (e.g. Ehleringer and Mooney 1978 and Givnish andVermeij 1976). Givnish (1987) reviewed 23 ecological patterns in leaf form, physiology and arrangement and the underlying trade-offs associated with each condition. Leaf hairs are thought to affect leaf physiological processes through a number of definable interactions (Fig. 3; Ehleringer and Mooney 1978). Leaf hairs tend to increase the thickness of the boundary layer (Fig. 3; e.g. Wuenscher 1970). An increase in boundary layer thickness will directly influence both photosynthesis and transpiration through its effect on restricting the diffusion of CO_2 into and water vapour from the leaf surface (Fig. 3; Ehleringer and Mooney 1978). In addition the thickness of the boundary layer will also have an effect on heat exchange from the leaf (or leaf temperature) (Fig. 3; Ehleringer and Mooney 1978 and Givnish 1987). Leaf temperature affects the saturation water vapour pressure which in turn is a determining factor of the transpiration rate (Ehleringer and Mooney 1978). Leaf temperature also affects photosynthesis, which is an enzyme controlled (and thus temperature dependent) reaction (Ehleringer and Mooney 1978). Therefore leaf hairs also have indirect effects on photosynthesis and transpiration (Fig. 3). Leaf hairs decrease light absorptance of the leaf (or increase light reflectance) thus allowing less light for photosynthesis (Fig. 3; Ehleringer and Mooney 1978). Decreased absorptance will also lower the leaf temperature, which will have indirect effects on both transpiration and photosynthesis as previously discussed (Ehleringer and Mooney 1978).

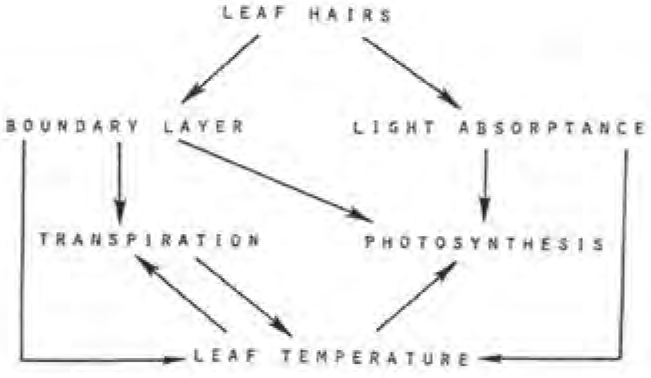


Fig. 3: Potential interactions between leaf hairs and leaf physiological activities (from Ehleringer and Mooney 1978).

Leaf size is also thought to have an affect on transpiration and photosynthesis (Fig. 4; Givnish 1987). As the width of a leaf increases the thickness of the boundary layer (the film of stagnant air surrounding the leaf surface) increases (Givnish 1987). An increase in thickness of the boundary layer will reduce transpiration and photosynthesis both directly and indirectly through a reduction in leaf temperature as outlined previously (Fig. 4; Ehleringer and Mooney 1978). An increase in leaf size also allows for a greater area over which light capture can take place, leading to an increase in absorptance (Fig. 4; Ehleringer and Mooney 1978)

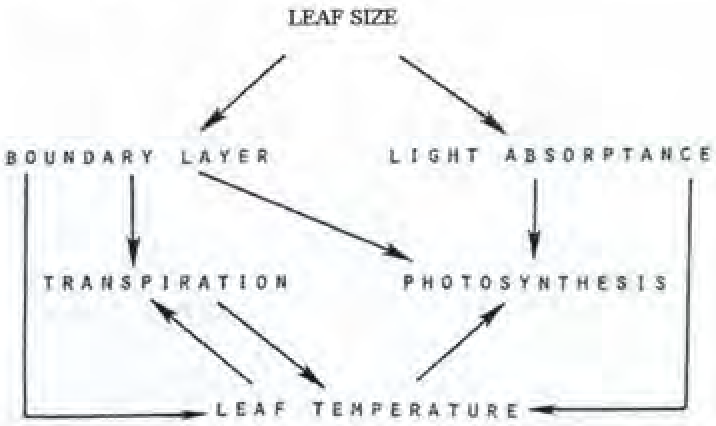


Fig. 4: Potential interactions between leaf size and leaf physiological processes (adapted from Ehleringer and Mooney 1978).

A number of studies have investigated changes in leaf properties along environmental condition gradients (Givnish 1987). Effective leaf size has been found to increase along gradients of increasing rainfall, humidity and/or soil fertility (Givnish and Vermeij 1976). However, Givnish and Vermeij (1976) also reported that there are fairly complex interactions between leaf size, water availability and radiation load. Leaves also tend to be more highly reflective or glaucous in more arid, sunnier and less fertile regions (Sandquist and Ehleringer 2003). Sandquist and Ehleringer (2003) conducted a population-level investigation into variation in leaf pubescence in the Brittlebush (*Encelia farinosa*, Asteraceae). They found that absorptance was always greatest for plants occurring in high-rainfall environments (Sandquist and Ehleringer 2003).

Pollination, adaptation and speciation:

Few studies (e.g. Ellis *et al.* 2006) have attempted to relate changes in leaf properties along environmental gradients back to speciation. One of the greatest challenges to evolutionary biologists is to understand the relationship between adaptation and speciation. Do populations become locally adapted to physical conditions and subsequently become isolated due to, for example, phenological differences? Or do populations initially become isolated due to selection for a different pollinator? Carson (1985) suggested that the determinants of speciation in plants are reflected in the patterns of radiation in either the floral or the vegetative characters. Johnson (1996) used this notion to explore the relative contributions of the physical and pollination environment in the diversification of Cape flora. When a family or genus, such as *Leucospermum*, has radiated primarily in vegetative characters it suggests adaptation to the physical environment is the main determinant of speciation (Johnson 1996). Alternatively when a family or genus, such as *Pelargonium*, displays remarkable diversity in floral form it suggests adaptation to the pollination environment as the main determinant of speciation (Johnson 1996). This simple method of distinguishing between floral and vegetative character radiation is a useful exploratory tool for inferring the environmental determinants of speciation (Johnson 1996). A more precise approach would be to compare patterns of character divergence among closely related species or even subspecies. The two subspecies of *L. conocarpodendron* provide an ideal opportunity to investigate the relative importance of the pollination and physical environment in potential speciation.

Aims and objectives:

The first objective of this study was to investigate whether there are any differences in vegetative properties between *L. conocarpodendron* ssp. *conocarpodendron* and *L. conocarpodendron* ssp. *viridum*. In particular the aim of this part of the study was to provide quantitative evidence of any differences in leaf form, size, reflectance in the photosynthetically active region (PAR: 400-700nm), hairiness, specific leaf area and stomatal density. Subsequently we aimed to evaluate the affects of these leaf traits on the transpiration rate. We predicted that transpiration rate would increase with an increase in stomatal density and that it would decrease with an increase in hairiness and leaf size. The second objective of the study was to relate our findings of the first objective back to speciation. In particular I wanted to investigate whether the leaf traits were adaptive and thus whether local adaptation to the physical environment was causing and maintaining the separation of the subspecies, as opposed to the pollination environment.

Specifically I asked the following questions:

1. Are there any quantifiable differences in leaf traits between the two subspecies?
2. What is the adaptive significance of these leaf traits?
3. What role do leaf traits have in maintaining subspecies boundaries?

Methods:

Study sites:

Long-term averaged climate variables for 4 sites along the peninsula were obtained using a model developed by Schultze (1999) in ArcView 3.3 (Table 1). The mean rainfall and mean daily maximum temperature for summer was calculated as being the average of the values for November, December and January, while the corresponding winter values were calculated as the average of the values for June, July and August. Soil data was obtained from a geological survey map - 3418 AB&AD - based on aerial photographs of the Cape Peninsula at a scale of 1: 50 000. Plants selected from each site for analysis were all greater than 1.5m tall to control for size-related effects and age-related effects.

Fig. 5: Geology and soils of the Cape Peninsula.

Reflectance:

Leaves were collected from 5 sites along the Cape Peninsula, 2 of which had the green phenotype, 2 the grey phenotype and one with individuals of intermediate phenotype. Leaf reflectance from the adaxial surface of each leaf in the PAR was measured within 24 hours of collection using an integrating sphere within an OceanOptics USB2000 spectrophotometer (OceanOptics.com).

Stomatal density and hairiness:

We applied nail-polish onto the adaxial surface of 5 leaves of each individual collected from each of the 5 sites. The nail polish film was subsequently peeled off and analysed under a compound microscope at 100X magnification. The stomatal density was calculated as being the number of stomata that occurred along a standard length of the eye-piece graticule. The hairiness of a leaf was measured by counting the number of hair pores occurring along the length of an eye-piece graticule. The mean hairiness and stomatal density of each individual was calculated as the total number of hairs or stomata found on each leaf divided by the number of leaves per individual.

Leaf temperature:

The temperature of the top (most exposed) 5 leaves from each stem used in the gravimetric analysis was recorded using a temperature gun immediately after the gravimetric analysis had been conducted.

Boundary layer:

Our measure of boundary layer thickness only considered variation in leaf size and not leaf hairiness, as leaf hairiness was analysed separately. The diameter of the largest circle that could be fitted onto each leaf was recorded for 5 leaves from each individual collected from 5 sites. Boundary layer was calculated from an equation developed by Nobel (1974):

$$B = 4 \text{ X } \sqrt{[D^{0.5}/ws]},$$

where B is the boundary layer, D is the diameter of the largest circle that can be fitted onto each leaf and ws is the wind velocity. Wind velocity was kept constant for each leaf and thus the equation was reduced to the following:

$$B = 4 X \sqrt{} (D^{0.5}).$$

Leaf area and specific leaf area (SLA):

Fresh leaves collected from each of the study sites were pressed and subsequently dried in a drying oven over a period of about two weeks. The leaf area of each leaf was then measured using a LI-3100 Area Meter (Li-Cor, inc. Lincoln, Nebraska USA). Each leaf was then weighed using a Mettler AE200 electronic balance. The SLA was found by dividing the leaf area by the leaf weight.

Transpiration rates:

Measurements of transpiration were done by gravimetric analysis. Stems of roughly the same size and number of leaves were collected from 6 individuals of each type located on Eastern facing slopes in Camps Bay and immediately placed in water. The stems were cut again under water to ensure that xylem cavitation was reduced and then placed in glass bottles. The effects of specific environmental variation was minimised by placing the stems in close proximity to each other, in an area receiving full sunlight and equal wind velocity and humidity. The weight loss from each transpiration chamber was recorded using an OHAUS Scout Pro 2000g electronic balance at 6 intervals over a period of an hour. The total leaf area from each stem was subsequently recorded using a LI-3100 Area Meter (Li-Cor, inc. Lincoln, Nebraska USA). Transpiration rate was calculated by converting the weight loss ($W_{t=0} - W_{t = \text{final}}$) to mmols and dividing this by the total leaf area multiplied by the time taken.

Phenology:

Flowering phenology was measured at 9 sites along the Cape Peninsula over a period of a week. Inflorescences were divided into 3 biologically relevant categories: bud (perianth with little or no yellow colouration and very early style and anther development); mature (perianth with bright yellow colouration; styles straightened) and old (perianth fading yellow colouration and all styles collapsed).

Statistical analysis:

All statistical analyses were done using Statistica 7. A students t-test for independent samples was used to analyze the differences in mean stomatal density, hairiness, leaf area, leaf temperature, SLA and boundary layer between green and grey individuals. Results were considered significant at the $P < 0.05$ level. Reflectance data were averaged into categories of wavelength ranges (450-500nm; 500-550nm; 550-600nm; 600-650nm; 650-700nm). The datasets of average reflectance from each wavelength category were analyzed using ANOVA (Statistica 7).

Table 1: Physical variables for sites along the Cape Peninsula. The phenotype found at each site is given in brackets next to the site name.

Sites	**Mean Rainfall (mm)**		**Mean Daily Max. Temp. (°C)**	
	Summer	**Winter**	**Summer**	**Winter**
Camps Bay (Grey)	33	182	23.3	15.7
Tafelberg Road (Grey)	46	206	23.7	13.3
Chapman's Peak (Green)	41	185	22	12.7
Red Hill (Green)	21	102	22.7	15.3

Results:

Temperature, rainfall and soil type:

Mean rainfall for both summer and winter was found to be greatest at the Tafelberg Road site (grey population) and lowest at the Red Hill site (green population) in both summer and winter (table 1). Mean summer rainfall was less than 50mm for all sites (table 1). Mean summer temperatures were greater at the two sites with the grey phenotype (Camps Bay and Tafelberg Road; table 1). There was found to be overlap in terms of the kind of soil that each phenotype occurs on (Fig. 5). The soil at the Red Hill (green) and Tafelberg Road (grey) sites consisted of grey to reddish quartzitic sandstone with minor grit, conglomerate and reddish shale lenses, gritty sand and dolerite at the Camps Bay site (grey) and dolerite at the Chapman's Peak site (green; Fig. 5).

Reflectance:

Across all wavelengths there was a significantly higher reflectance from glaucous (grey) than green leaves ($F_{5, 14} = 15.823$; $p = 0.00003$; Fig.6). The grey individuals reflected about 6% more light than green individuals for wavelengths ranging from 450-600nm, 9% more light between 600nm and 650nm and about 7% more light between 650nm and 700nm (Fig. 6).

Leaf temperature:

Green leaves had a higher temperature than grey leaves, although this was not statistically significant ($t = -2.006$; d.f. = 10; $p = 0.072$). The mean leaf temperature of grey individuals was found to be 28.17˚C, while this value rose to 30.26˚C for green individuals (Fig. 7).

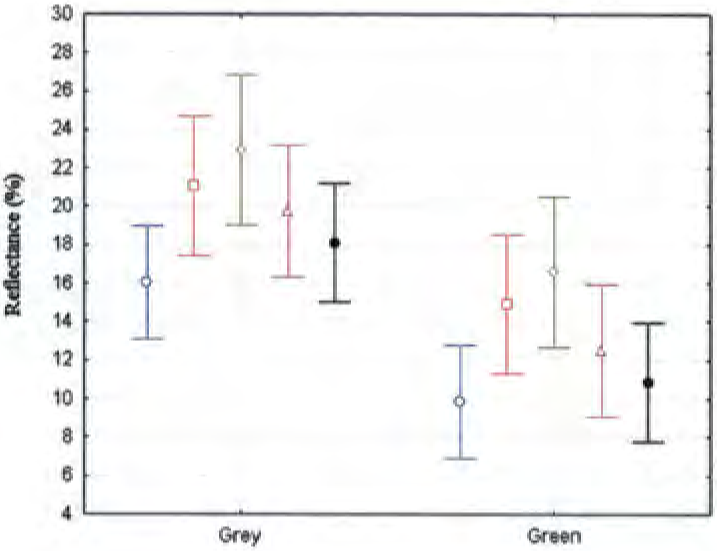


Fig. 6: Comparison between the mean reflectance values for wavelength categories for green and grey leaves. The blue circles correspond to a wavelength category of 450-500nm, red squares = 500-550nm, green diamonds = 550-600nm, pink triangles = 600-650nm and black circles = 650-700nm. The vertical bars denote 95% confidence intervals.

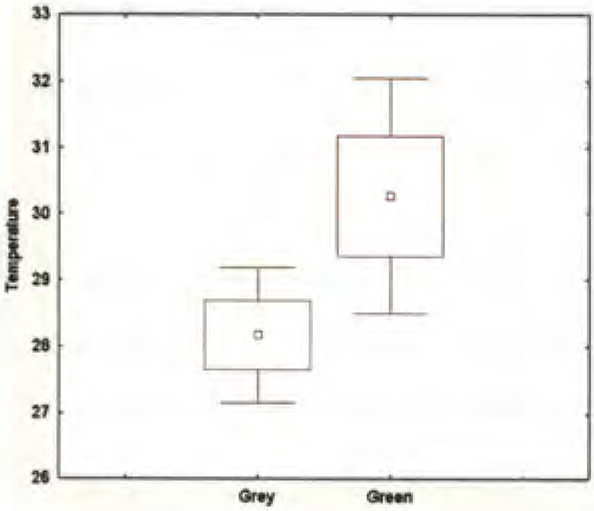


Fig. 7: Comparison between green and grey individuals in terms of the leaf temperature, showing the mean value of six individuals of each phenotype and standard error bars.

Stomatal density:

We found a significant difference between the stomatal density of green and grey individuals ($t = 3.33$; d.f. = 58; $p = 0.0015$; Fig. 8). The mean number of stomata for green individuals was found to be 11.37, while this value rose to about 13.6 in grey individuals (Fig. 8). This trend was also observed in individuals taken from a number of sites from around the peninsula ($t = 4.044$; d.f. = 98; $p = 0.0001$).

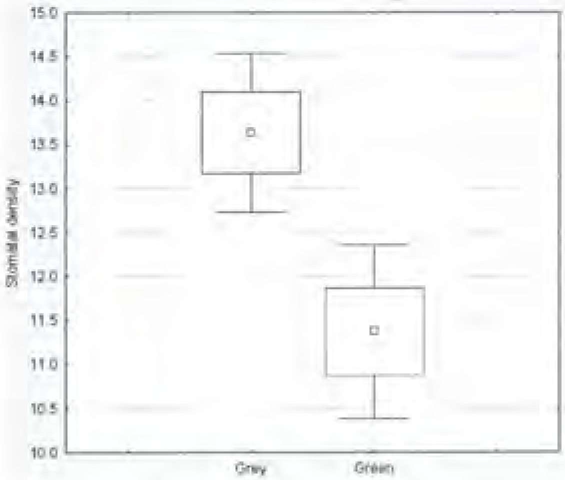


Fig. 8: Comparison between green and grey individuals in terms of stomatal density, showing the mean stomatal density for 6 individuals of each phenotype and standard error bars.

Boundary layer:

We found that green individuals generally had a significantly larger boundary layer than grey individuals ($t = -3.62$; d.f. = 58; $p = 0.0006$; Fig. 9). The mean boundary thickness for green individuals was found to be approximately 9.5 and 9.2 for grey individuals (Fig. 9).

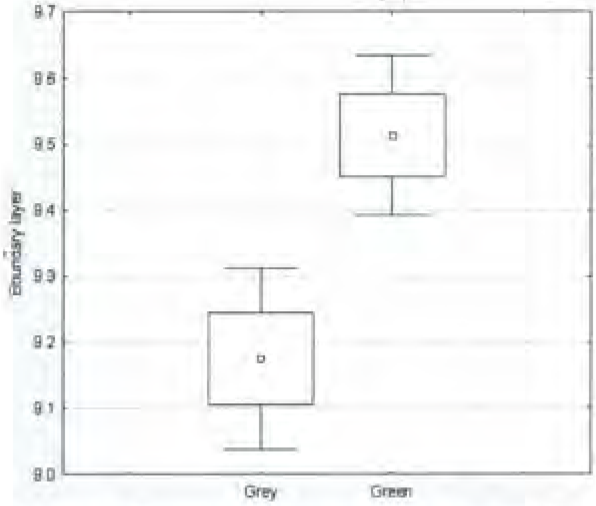


Fig. 9: Showing the difference between green and grey individuals in terms of the mean boundary layer (E) with standard error bars.

Leaf area and SLA:

The leaves from green individuals were found to have a larger area than those from grey individuals (t = -3.86; d.f. = 58; p = 0.0003; Fig. 10). The mean area of green leaves was found to be 21.16 cm^2, while this was only 15.95 cm^2 in grey individuals. The median value for green individuals was 19cm^2 and 15cm^2 for green and grey individuals respectively, indicating that there were a number of green leaves with particularly large leaf area. The green leaves were also found to have higher SLA values than grey leaves (t = -6.722; d.f. = 58; p = 0.000; Fig. 11). The mean SLA was found to be 41.9 cm^2/g for green leaves and 35.7 cm^2/g for grey leaves (Fig. 11).

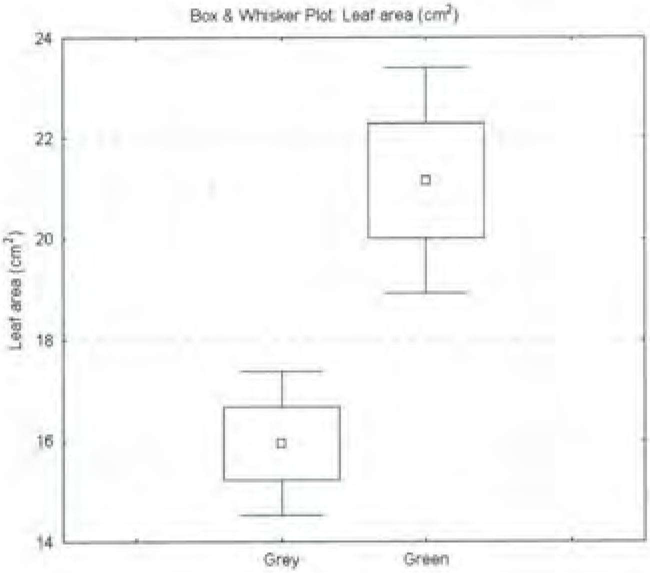


Fig. 10: The difference in leaf area between green and grey individuals from a number of sites around the peninsula. The mean values for each type are represented, along with standard error bars.

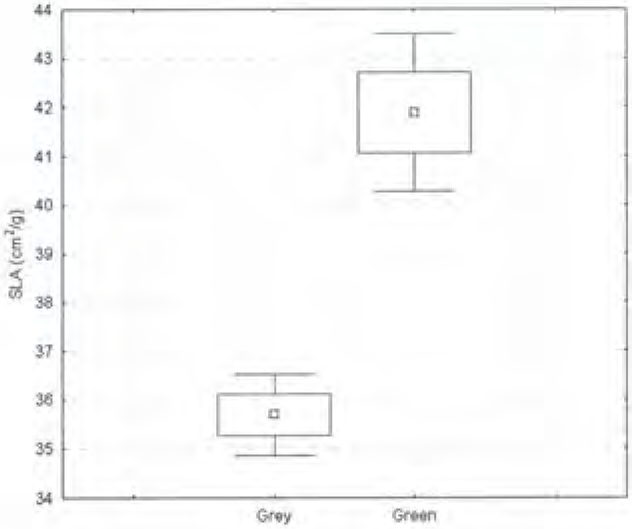
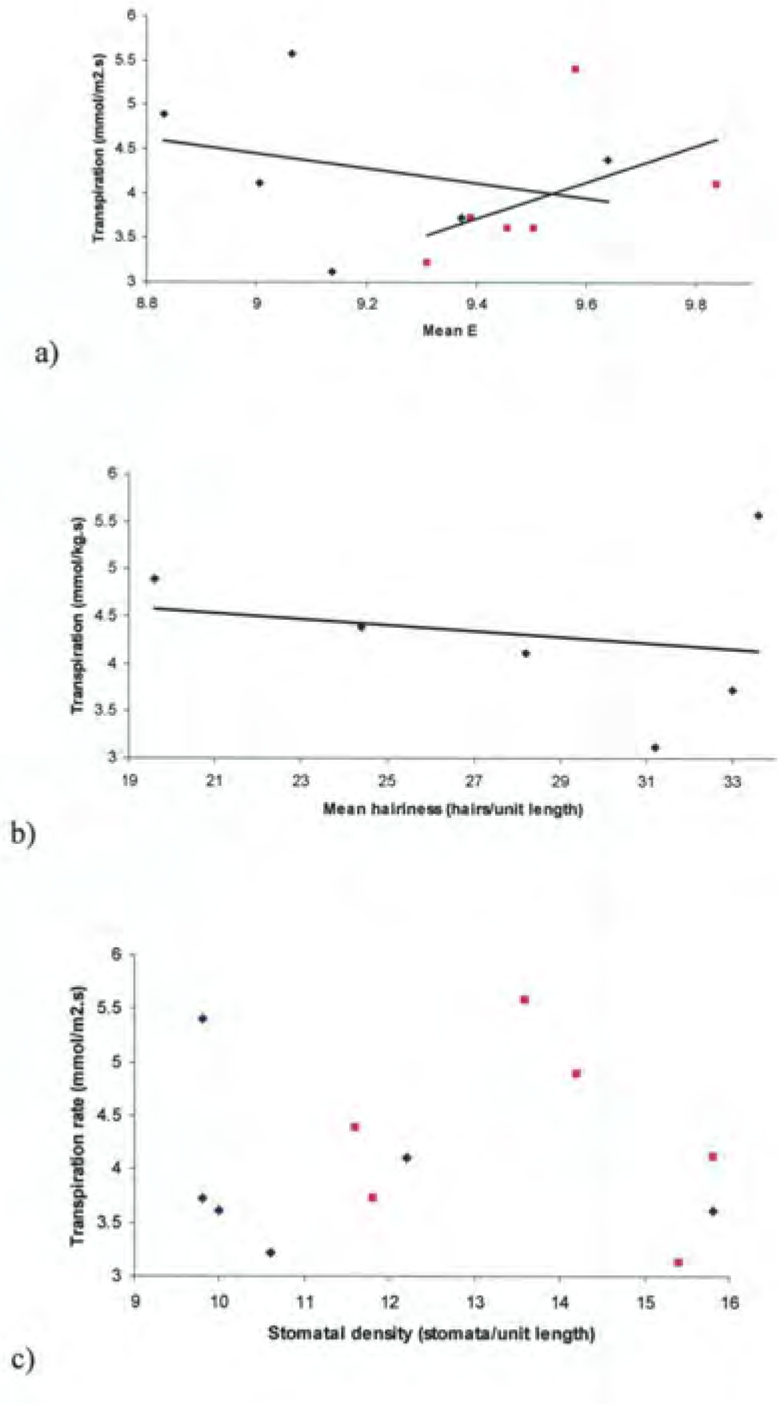


Fig. 11: The difference in SLA between green and grey leaves, showing standard error values.

Transpiration rate and leaf traits:

There was no correlation between the size of the boundary layer (E) and transpiration rate ($p = 0.14$; Fig. 12a). There was no significant correlation between boundary layer size and transpiration rate within each phenotype either (Fig. 12). No correlation was found between hairiness and transpiration rate among grey individuals ($p = 0.2$; Fig. 12b) nor between mean stomatal density and transpiration rate ($p = 0.05$; Fig. 12c). No correlation was found between mean stomatal density and transpiration rate within each phenotype (Fig. 12c).



<u>Fig. 12: The relationship between transpiration rate and leaf traits. a.) shows the relationship between size of the boundary layer and transpiration rate; b.) shows the relationship between mean hairiness for grey individuals and transpiration rate and c.) shows the relationship between stomatal density and transpiration rate</u>

Flowering phenology:

Our results show that green individuals were in an advanced state of flowering when compared to the grey individuals (Fig. 13). The majority (92%) of inflorescences on grey individuals were not fully developed, while only about 8% were mature (Fig. 13). While the majority (54%) of inflorescences were also juvenile in green individuals, a significant proportion were either fully mature (30%) or already past maturity (16%; Fig. 13).

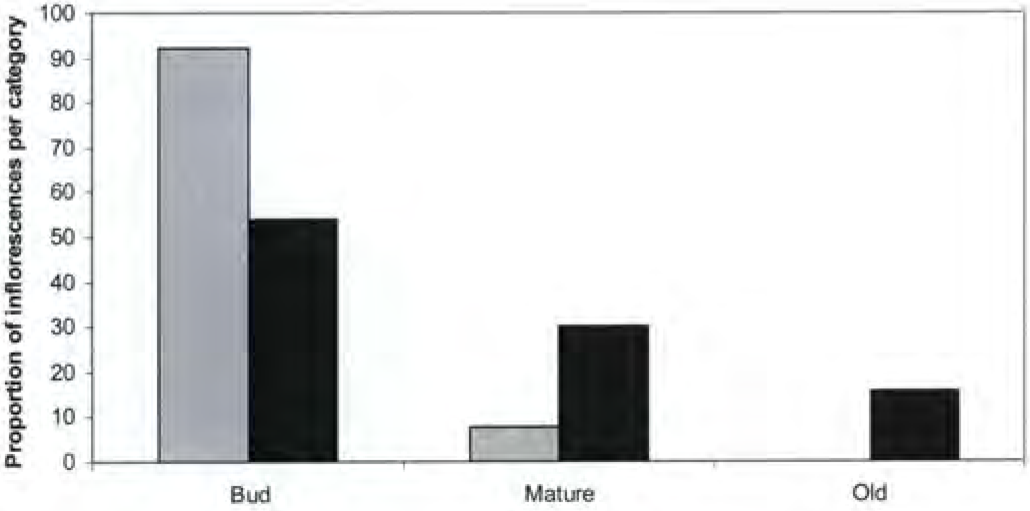


Fig. 13: The proportional number of inflorescences that were buds, mature or old in green (black bars) and grey (grey bars) individuals.

Discussion:

Our study has shown that there are significant differences between the grey and green phenotypes in important leaf characteristics, such as stomatal density, SLA, hairiness and leaf size. What is the adaptive significance of these differences?

Leaf traits and transpiration:

A number of the correlations between transpiration rate and leaf traits are somewhat surprising. The finding that stomatal density has no effect on transpiration in both green and grey individuals is contrary to our initial prediction that an increase in stomatal density will lead to an increase in transpiration (Martin *et al.* 1999). Stomatal conductance is known to be a function of the density, size and degree of opening of stomata (Martin *et al.* 1999). Stomata have been found to close during periods of high temperature or low humidity (Tenhunen *et al.* 1984). Our gravimetric analysis was conducted between 13h00 and 15h00, when the temperature would have been close to the daily maximum on a particularly warm day in October. It is possible that the stomata were not fully opened during the gravimetric analysis, which could have lowered the transpiration rate of individuals with high stomatal density.

That leaf hairiness had little to no effect on transpiration rate suggests that hairs have little or no effect on the thickness of the boundary layer in *L. conocarpodendron* ssp. *conocarpodendron*, although we are reluctant to draw any definite conclusions from these correlations considering the surprising lack of a correlation between stomatal density and transpiration rate. This finding is supported by Ehleringer and Mooney (1978) who reported that the impact of leaf hairs of *Encelia farinosa* on the thickness of the boundary layer is small. Their results led them to conclude that the effect that hairs have on the boundary layer will only have a small effect on transpiration and an even smaller effect on the total resistance to CO_2 transfer for photosynthesis (Ehleringer and Mooney 1978). These findings are contrary to those of Wuenscher (1970) which found that leaf hairs in *Verbascum thapsus* increased the boundary layer. Wuenscher (1970) mentioned that different types of hairs affect leaves in different ways and that some hairs may have no significant function. It is possible that the hairs on *L. conocarpodendron* ssp.

conocarpodendron serve a different function other than increasing the boundary layer and reducing the transpiration rate.

Leaf traits, rate of photosynthesis and growth strategies:

A number of studies have found that leaf hairs may have direct and indirect effects on the rate of photosynthesis (e.g. Ehleringer and Mooney 1978). One might expect a decrease in the rate of photosynthesis with an increase in reflectance as there is less light available for photosynthesis (Ehleringer and Mooney 1978). In environments with high daily maximum temperatures an increase in reflection may lower the leaf temperature to values closer to that of the optimal photosynthetic temperature, which may serve to increase the rate of photosynthesis (Ehleringer and Mooney 1978). Ehleringer and Mooney (1978) suggested that there are two options for maintaining low leaf temperatures relative to air temperatures in these hot environments: either the plant has to invest heavily in leaf pubescence to reduce leaf absorptance or have a high leaf conductance to increase the cooling effects of evapotranspiration. Ehleringer and Mooney (1978) suggested that in environments where water is also a limiting resource, the former strategy may be better as this ensures that a lower amount of energy is needed to be dissipated through transpiration in order for the leaf to remain at a cooler temperature. The relationship between water vapour pressure and temperature is exponential (Ehleringer and Mooney 1978) which implies that the amount of water conserved may be substantial at moderate to high temperatures.

This information combined with our results allowed us to develop a hypothesis concerning potential light capture and growth strategies of green and grey individuals. We propose that green individuals may be adapted to take advantage of conditions at an earlier stage in the growing season than grey individuals. On the other hand green individuals may be less well adapted to cope with stressful conditions, such as those experienced during the height of summer when temperatures are highest and rainfall is lowest (table 1). The strategy of green individuals may involve having a greater photosynthetic rate during the cooler winter months but then "shutting down" over stressful periods when conditions are unsuitable to maintain high transpiration and photosynthetic rates.

This suggestion is supported by our finding that grey individuals reflect more light than green individuals (Fig. 6). By reflecting light in the PAR grey individuals may reflect otherwise useful quanta reducing their rate of photosynthesis. Grey individuals may thus take longer to achieve the same level of photosynthesis as green individuals during the early summer period. This suggestion is further supported by our finding that green individuals have a greater SLA than grey individuals (Fig. 11). An increase in SLA has been shown to correlate with higher percentage leaf nitrogen levels and a faster photosynthetic rate (Wright *et al.* 2004).

While grey individuals may take longer to achieve the same rate of photosynthesis they may also be able to persist for longer during periods of relative stress. A number of studies have shown that at any given light intensity unfavourable abiotic conditions such as high temperature (e.g. Paolacci *et al.* 1997) and drought (e.g. Munne-Bosch *et al.* 2001) will limit photosynthetic light energy utilization. This increases the proportion of excess light energy absorbed and thus of photoinhibition (Close *et al.* 2007). The Cape Peninsula has a winter rainfall pattern, and receives less than 50mm of rainfall during summer when temperatures are highest (table 1). By increasing reflectance from their leaves, grey individuals may be able to lower the risk of photoinhibition during these times and keep photosynthesizing for longer.

Our suggestion is further supported by the flowering phenology data, which shows that green individuals are able to flower earlier than grey individuals (Fig. 13). This indicates that the green individuals are able to allocate more resources into reproduction at an earlier stage in the growing season.

In summary the two different growth strategies are to have either a shorter but much faster growing season or a relatively slow but longer growing season. Grey individuals, because of increased reflectance from the leaves are able to persist throughout summer in hot, dry conditions, although they may suffer the cost of reduced photosynthetic rate during late winter and early summer when conditions are relatively mild. Green individuals are able to start growing earlier due to increased rate of photosynthesis but are not able to persist for as long as the grey individuals during mid to late summer. There are a number of ways in which one would go about testing this hypothesis. One could measure the photochemical efficiency using a flourometer over a range of seasons. This would allow one to analyze whether and how the rates of photosynthesis differ seasonally. If our hypothesis is correct one would expect the green individuals to display greater photosynthetic efficiency than grey individuals during early summer when conditions are mild, as the grey individuals would reflect more quanta that might otherwise be used in photosynthesis. We also predict that this trend would be reversed during mid to late summer as grey individuals would reflect more light from their leaves than green individuals, and therefore would have lower leaf temperatures, which would allow them to photosynthesize at a higher rate. During the course of this study we observed green individuals growing in Camps Bay. This would allow future investigations to compare the rate of photosynthesis while controlling for environmental variables like radiation and temperature.

In addition one could measure the relative growth of seedlings in a recently burnt environment over a number of seasons. If our hypothesis is correct green individuals might be expected to grow faster during early summer periods and to slow down as the summer progresses. Grey individuals on the other hand would be expected to show slow growth during early summer but would increase in size as the summer progressed. One could also measure the phenology of flowering over a longer period than our study allowed for. If our hypothesis is correct one would expect green individuals to flower earlier than grey individuals but for grey individuals to flower for longer periods.

A note on the driving force behind a possible speciation event:

Our study provides quantitative evidence that there is considerable variation in vegetative properties in *Leucospermum conocarpodendron*. In accordance with Johnson's (1996) method of distinguishing between floral and vegetative character radiation to infer the likely environmental determinants of speciation, this suggests that a potential speciation event between the two subspecies is being driven by adaptation to edaphic environment. However, our data also suggests that adaptation to edaphic environment may be associated with subsequent shifts in flowering phenology. A coupling of these two potentially isolating forces has been reported previously by Ellis *et al.* (2006). They investigated the evolutionary radiation of "stone plants" in the genus *Argyroderma* and reported that flowering time shifts were associated with morphological adaptations to different local habitats (Ellis *et al.* 2006).

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