Temperate Zones: Investigating Convergence versus Non-convergence

Summary

Variations in plant morphology are useful for assessing broad questions such as convergent versus nonconvergent evolution across plant species. A range of measurements were collected from plant species in the Temperate House at Kew Royal Botanical Gardens, including measurements of leaf length-to-width ratio, leaf area, and leaf waxiness. Statistical analyses revealed significant differences in these traits among continents, with Africa and Western Australia showing distinct leaf length-to-width ratios, possibly due to climate variations. Additionally, differences in leaf area were observed between Western Australia and other regions, suggesting adaptations to arid conditions. The Americas exhibited unique leaf waxiness, likely influenced by biotic and abiotic stressors. Understanding these trait variations is crucial for predicting plant responses to environmental changes. Further research should explore the underlying mechanisms driving these variations, contributing to a deeper understanding of plant ecology and adaptation in diverse biogeographic contexts.

Keywords

Introduction

The field of comparative plant morphology posits that limited optimal adaptive solutions in similar climatic zones lead to high levels of convergent evolution among organisms (Cody and Mooney, 1978). However, this theory faces disputes due to various factors such as migration, extinction, and speciation (Verdú *et al.*, 2003). Additionally, similarities previously attributed to convergent evolution in Mediterranean lineages were revealed to be influenced by phylogenetic and historical constraints (Verdú *et al.*, 2003).

This study seeks to evaluate differences in plant traits across temperate biogeographic regions. With a hypothesis centred on evolutionary convergence, we anticipate observing similar trait averages and variances among plants across different temperate regions.

Materials and Methods

We randomly sampled and obtained quantitative and qualitative measurements from plant species in Temperate House, Kew Royal Botanical Gardens (RBG Kew), Richmond in February 2024. For each individual, we measured the length and width of its leaves and scored them on 7 different qualitative characters as outlined in Table 1. Each quantitative measurement was repeated three or more times and an average was produced.

Observation	Description	Measurement
Species	Binomial name of plant	Species name
Continent	The plant's native temperate region	Native floristic zone (1-7†)
Leaf.LW	The ratio of leaf length to leaf width for each individual	3-5 Numerical measurements (mm)
Leaf.abs	Absolute area (L*W) of each leaf	3-5 Numerical measurements (mm²)

Observation	Description	Measurement
Serrated.Smooth	Presence (1) or absence (0) of serrated edges on leaves	Presence/Absence
Hairy.Non.hairy	Presence (1) or absence (0) of hair or fuzz on leaves	Presence/Absence
Waxy.Non.waxy	Presence (1) or absence (0) of waxy coating on leaves	Presence/Absence
Compound.Simple	Presence (1) or absence (0) of compound leaf structure	Presence/Absence
Central.Auxiliary	Presence (0) or absence (1) of auxiliary structures (e.g. stipules)	Presence/Absence

Table 1: Outline of the data structure. Each individual's binomial name and native temperate region was recorded based on its associated label in the Temperate House at RBG Kew. The 7 temperate zones† include Africa, the Americas, Asia, the Himalayas, Australia, Western Australia, and New Zealand.

Statistical analyses were carried out in R version 4.1.2. PCA was used to assess which combinations of variables contributed most significantly to the morphological differences between temperate zones. Before conducting PCA, the data underwent preprocessing, which involved computing the mean leaf lengths and widths for each plant species. Subsequently, two metrics were derived from the separate measurements of leaf length and width: the leaf length-to-width ratio (leaf length: width) and absolute leaf size (leaf length*width). These metrics were chosen to comprehensively capture species-specific morphological diversity, with leaf length: width emphasizing shape-related characteristics and leaf length*width focusing on size-related traits. Notably, outlier length and width measurements were retained, as they did not compromise the integrity or robustness of the data analysis.

Following visual inspections of the PCA biplots and other diagnostic plots, leaf length: width and leaf waxiness were the only traits identified as heteroscedastic variables, and were subsequently bootstrapped for additional rigor. We fitted linear models of the different traits against temperate zones. ANOVAs conducted across the individual traits identified leaf waxiness and the correlated measurements of leaf length and leaf width to be key defining characteristics which differentiate plants from different temperate zones.
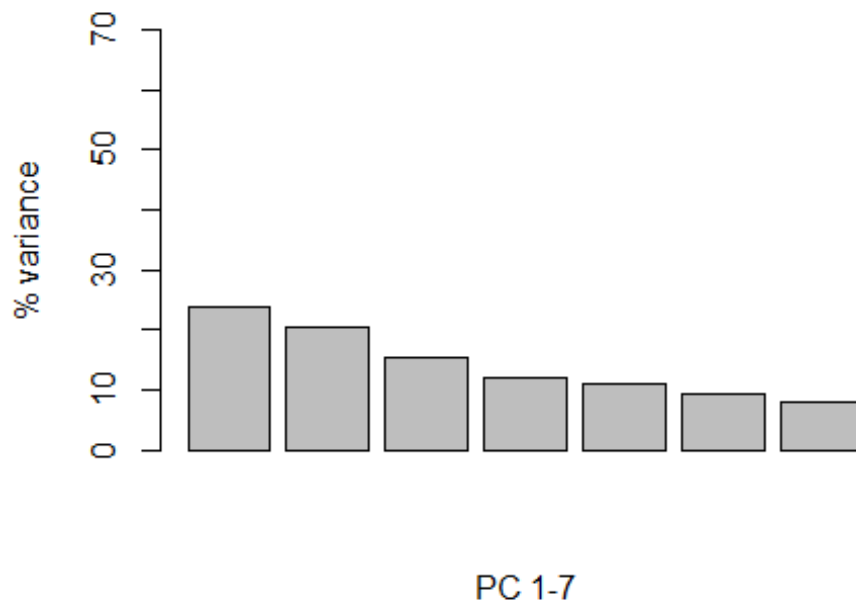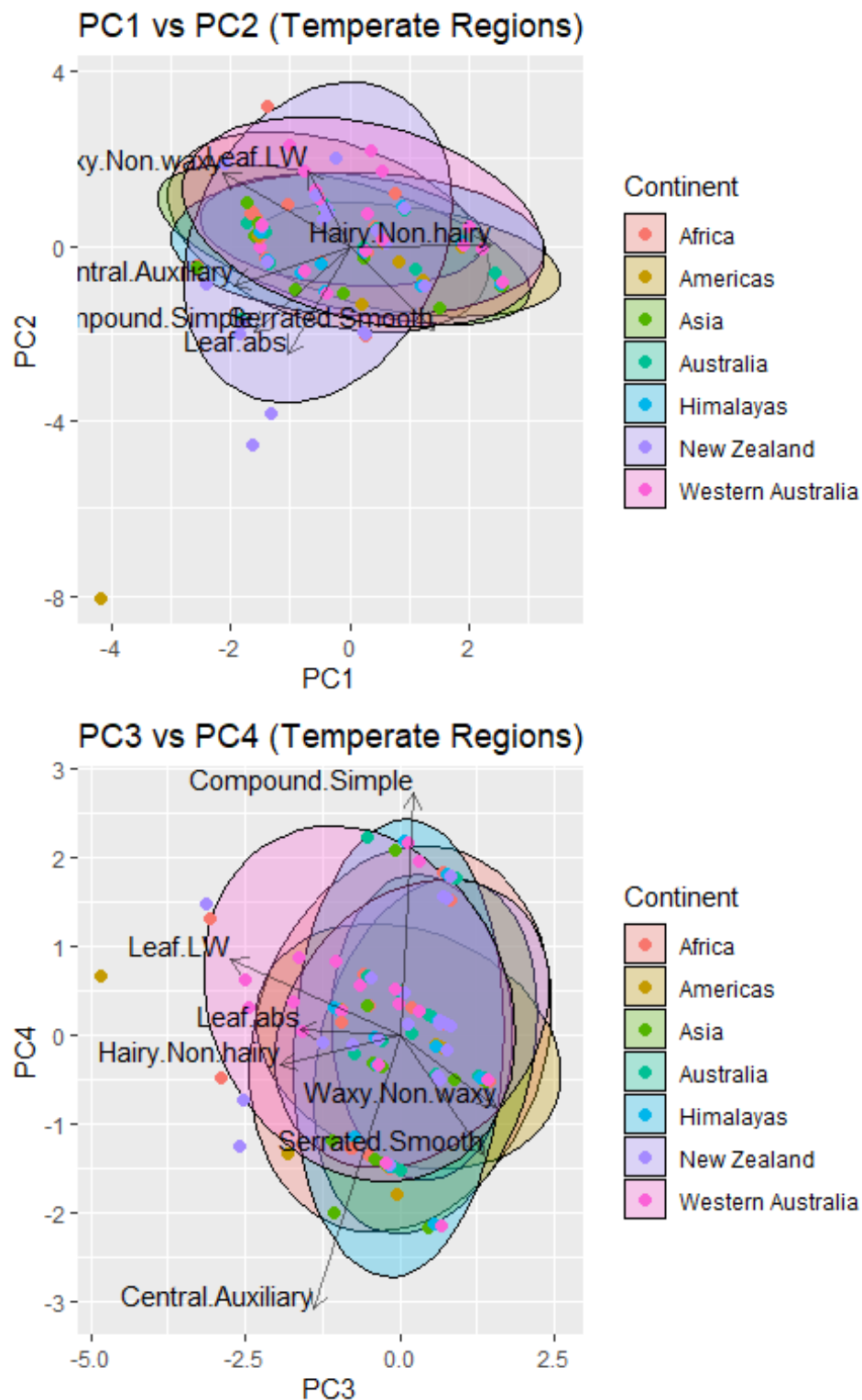
Results

Figure 1: *The first 4 principal components capture approximately 70% of the data, with PC1, at 23%, this is followed by PC2 (20%), PC3 (15%) and PC4 (12%).*

Figures 2 and 3: *Principal component analysis (PCA) biplots for the trait dataset.*

```
##              Df  Pillai approx F num Df den Df    Pr(>F)
## Continent    6 0.29712    2.0194       24     604 0.002943
## Residuals 151
```

Table 2: MANOVA test result for principal components 1 to 4 (PC1-PC4) across continents.

A MANOVA test (Table 2) was used to examine whether there are significant differences among biogeographical zones ("continents") in the multivariate response variables (Figures 2 and 3). Approximately 29.71% of trait variance was explained by the Continent variable. The p-value indicates that there is significant evidence to reject the null hypothesis of no group differences (p<0.05).

```
## Analysis of Variance Table
##
## Response: Serrated.Smooth
##             Df Sum Sq Mean Sq F value Pr(>F)
## Continent    6  2.019 0.33646  1.5154 0.1767
## Residuals 151 33.526 0.22202
## Analysis of Variance Table
##
## Response: Hairy.Non.hairy
##             Df  Sum Sq Mean Sq F value Pr(>F)
## Continent    6  1.5937 0.26561  1.6361 0.1409
## Residuals 151 24.5139 0.16234
## Analysis of Variance Table
##
## Response: Compound.Simple
##             Df  Sum Sq Mean Sq F value Pr(>F)
## Continent    6  1.0872 0.18121  1.3711 0.2298
## Residuals 151 19.9571 0.13217
## Analysis of Variance Table
##
## Response: Central.Auxiliary
##             Df  Sum Sq Mean Sq F value Pr(>F)
```

```
## Continent   6  1.0013 0.16689  0.9601 0.4543
## Residuals 151 26.2455 0.17381
```

Table 3: ANOVA scores for Leaf Smoothness, Leaf Hairiness, Compound/Simple structure, and Central/Auxiliary structure show that none of these traits differed significantly across continents.

The absence of significant differences in traits such as the presence of serrations and hair on leaves, compound versus simple structure, and central versus auxiliary structure across temperate zones (p>0.05, Table 3) suggests uniformity in these characteristics among plant species within these regions. Moreover, the lack of significant differences in trait means across continents implies a comparable trait variance across temperate zones.
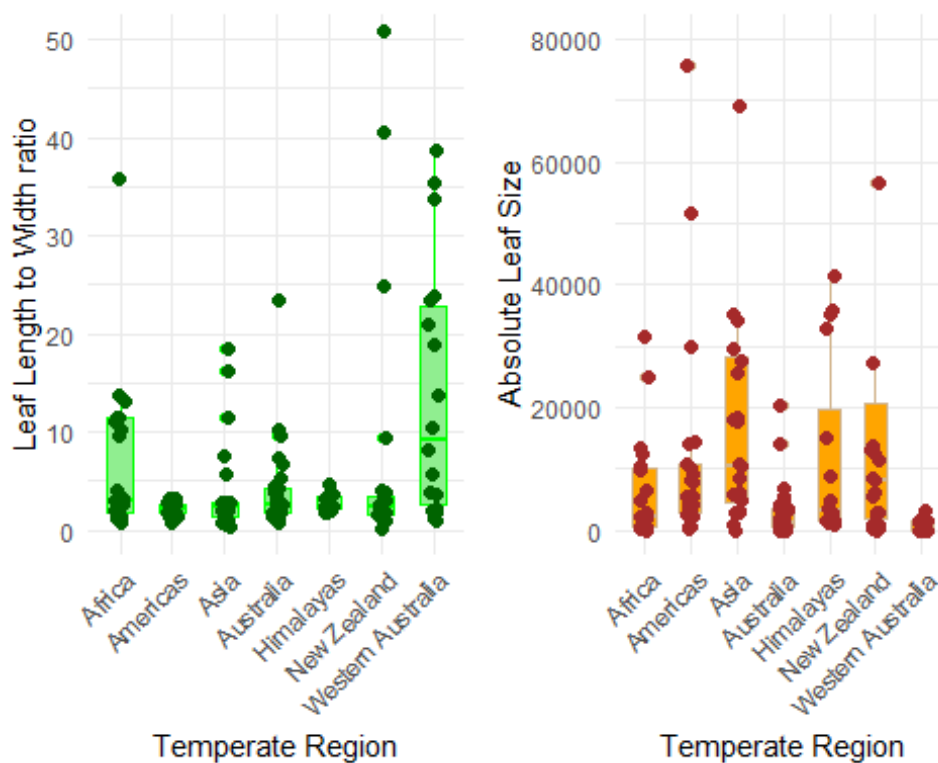

Figure 4: Boxplots illustrate the average leaf length: width (green) and absolute leaf area (orange) across continents. To facilitate clearer visualization and aid data interpretation, the plot was adjusted to display a narrower range of values along the y-axis.

```
##       Source Df   Sum_Sq   Mean_Sq  F_value Pr
## 1 Continent  6 2.006607 0.3344345 1755.487  0
```

Table 4: The ANOVA score shows that plant species differ significantly in their Leaf Width: Length ratio across continents, with a very low p-value. Leaf width: length ratios in this analysis were bootstrapped for additional rigour.

```
## Analysis of Variance Table
##
## Response: log(Leaf.abs)
##              Df Sum Sq Mean Sq F value    Pr(>F)
## Continent     6 263.33  43.888  10.969 3.984e-10
## Residuals   151 604.15   4.001
```

Table 5: The ANOVA score shows that plant species differ significantly in their absolute leaf area across continents, with a very low p-value.

```
##       Source Df   Sum_Sq   Mean_Sq  F_value Pr
## 1 Continent   6 2.517097 0.4195161 3315.224  0
```

Table 6: The ANOVA score shows that plant species differ significantly in whether they are waxy or non-waxy across continents, with a very low p-value. Waxiness data in this analysis was bootstrapped for additional rigour.

The traits leaf length: width and leaf area (Figure 5) in addition to leaf waxiness show significant differences across continents (p < 0.05, Tables 4, 5, 6). This suggests that plant species in temperate zones have evolved differently in terms of their area, leaf shape and waxiness. The significant differences in trait mean across continents may also imply differences in trait variances, as the variance contributes to the mean differences observed.

```
## (a)
##
##                 Africa  Americas Asia    Australia Himalayas New Zealand
## Americas        0.12393 -         -       -         -         -
## Asia            0.87435 1.00000   -       -         -         -
## Australia       0.94747 1.00000   1.00000 -         -         -
## Himalayas       0.55245 1.00000   1.00000 1.00000   -         -
```

```
## New Zealand       1.00000 1.00000  1.00000 1.00000   1.00000    -
## Western Australia 1.00000 0.00073  0.01030 0.01025   0.00858   0.42039

## (b)
##
##                   Africa Americas Asia    Australia Himalayas New Zealand
## Americas          0.9008 -        -       -         -         -
## Asia              0.1081 1.0000   -       -         -         -
## Australia         1.0000 0.0198   0.0011  -         -         -
## Himalayas         1.0000 1.0000   1.0000  0.2166    -         -
## New Zealand       0.6810 1.0000   1.0000  0.0153    1.0000    -
## Western Australia 0.0016 2.7e-07  8.6e-09 0.0636    2.6e-05   2.6e-07

## (c)
##
##                   Africa Americas Asia   Australia Himalayas New Zealand
## Americas          0.179  -        -      -         -         -
## Asia              1.000  0.027    -      -         -         -
## Australia         1.000  0.024    1.000  -         -         -
## Himalayas         1.000  1.000    1.000  1.000     -         -
## New Zealand       1.000  0.261    1.000  1.000     1.000     -
## Western Australia 1.000  1.000    1.000  1.000     1.000     1.000
```

Table 7: Pairwise t-test results for (a) Leaf.LW, (b) Leaf.abs and (c) Waxy.Non.waxy with Boneforroni adjustments.

Pairwise analyses (Table 7) compare the effect of different continents on the variable Leaf.LW (a), Leaf.abs (b) and Waxy.Non.waxy (c), and present p-values for pairwise comparisons between them.

Discussion

Comparisons of Leaf Morphology and Waxiness Across Continents

The findings of our study reveal significant variations in leaf morphology and waxiness across different continents, shedding light on the diverse ecological adaptations of plant

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species to their respective environments. Here, we discuss the implications and possible explanations for the observed differences in leaf traits.

Leaf Length-to-Width Ratio

Our analysis identified Africa and Western Australia as regions with particularly significant differences in leaf length-to-width ratios. These disparities may be attributed to distinct climate regimes and environmental conditions. Africa, characterized by a range of biomes and climates, exhibits diverse vegetation types adapted to varying temperature and moisture levels (Welk, Welk and Bruelheide, 2014). In contrast, Western Australia's arid climate and limited water availability may select for plant species with narrower leaves to reduce water loss through transpiration (Wright *et al.*, 2017; Fritz, Rosa and Sicard, 2018). Furthermore, differences in plant species composition and ecological pressures between the two regions could contribute to variations in leaf morphology.

Leaf Area

Significant differences in leaf area were observed between Western Australia and other regions within Australia. The arid conditions prevalent in Western Australia likely exert selective pressure on native plant species, favouring smaller leaf areas as an adaptation to conserve water (Ekwealor *et al.*, 2019). Additionally, the diverse vegetation types in Western Australia, ranging from shrublands to desert landscapes, may further influence leaf size variation (Wright *et al.*, 2017). The observed differences underscore the importance of water availability and vegetation type in shaping leaf area across geographical regions.

Leaf Waxiness

The Americas exhibited particularly significant differences in leaf waxiness compared to other continents. This could be attributed to a combination of biotic and abiotic stressors prevalent in the region. Plants in the Americas may have evolved higher leaf waxiness as a defence mechanism against herbivores, pathogens, and environmental stressors, such as high temperatures and drought (Shepherd and Wynne Griffiths, 2006). Additionally, the

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diverse range of plant species and ecological niches in the Americas (Shepherd and Wynne Griffiths, 2006; Echeverría-Londoño *et al.*, 2018) may contribute to variations in leaf waxiness as a result of adaptive evolution.

Discussion

The lack of significant differences in some traits supports the idea of convergent evolution in temperate zones, where plants may have evolved similar traits despite their geographic dispersion. However, the presence of significant differences in the three traits: leaf length-to-width ratio, leaf area, and waxiness, underscores the complexity of evolutionary dynamics within temperate zones. These differences suggest that additional factors beyond broad environmental similarities contribute to the divergence of certain traits among plant populations.

One possible explanation for these divergent trait adaptations is the influence of localized environmental conditions. While temperate regions share general climatic characteristics, subtle variations in factors such as soil composition, precipitation patterns, and microclimate can exert selective pressures unique to specific geographical locations (Tilman and Lehman, 2001). Consequently, plant populations may undergo divergent evolution to better suit their local ecological niches, resulting in the observed differences in traits like leaf morphology and waxiness.

Furthermore, historical factors, including past geological events and dispersal patterns, may have shaped the evolutionary trajectories of plant species in different temperate zones. Dispersal limitations and historical biogeographic events could have led to genetic isolation and subsequent divergence among plant populations, driving the evolution of distinct trait adaptations over time (Verdú *et al.*, 2003; Anacker and Strauss, 2014).

Moreover, interactions with other biotic factors such as herbivores, pathogens, and mutualistic organisms may also contribute to the divergence of certain traits among temperate plant species. Co-evolutionary dynamics between plants and their biotic counterparts can drive the selection for specific traits that enhance survival, reproduction,

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or defence, further accentuating differences among populations (Welk, Welk and Bruelheide, 2014).

Implications and Future Directions

Understanding the factors driving variations in leaf traits across continents is crucial for predicting how plant communities will respond to environmental changes, including climate change and land use alterations (Tilman and Lehman, 2001). Future research could delve deeper into the specific mechanisms underlying the observed differences, such as genetic adaptations, physiological responses to environmental cues, and interactions with other biotic and abiotic factors. Incorporating fine-scale environmental data and species-specific analyses would enhance our understanding of the ecological drivers of different plant characters at a greater scale.

Conclusions

The outcomes of our study underscore the complex interplay between environmental factors, plant adaptations, and geographical distributions in shaping leaf traits. By identifying these patterns, we contribute to a broader understanding of plant ecology and provide insights into the resilience and vulnerability of plant species in the face of changing environmental conditions. These findings underscore the importance of considering geographic context when studying plant traits, emphasizing the need for further research to elucidate the mechanisms driving trait variation and adaptation in diverse biogeographic contexts.

Word count: 1377

References

Anacker, B.L. and Strauss, S.Y. (2014) 'The geography and ecology of plant speciation: range overlap and niche divergence in sister species', *Proceedings of the Royal Society B: Biological Sciences*, 281(1778), p. 20132980. Available at: https://doi.org/10.1098/rspb.2013.2980.

Cody, M.L. and Mooney, H.A. (1978) 'Convergence Versus Nonconvergence in Mediterranean-Climate Ecosystems', *Annual Review of Ecology and Systematics*, 9(1), pp. 265–321. Available at: https://doi.org/10.1146/annurev.es.09.110178.001405.

Echeverría-Londoño, S. *et al.* (2018) 'Plant Functional Diversity and the Biogeography of Biomes in North and South America', *Frontiers in Ecology and Evolution*, 6. Available at: https://doi.org/10.3389/fevo.2018.00219.

Ekwealor, K.U. *et al.* (2019) 'Adaptive Strategies of Desert Plants in Coping with the Harsh Conditions of Desert Environments: A Review', *International Journal of Plant & Soil Science*, pp. 1–8. Available at: https://doi.org/10.9734/ijpss/2019/v31i530224.

Fritz, M.A., Rosa, S. and Sicard, A. (2018) 'Mechanisms Underlying the Environmentally Induced Plasticity of Leaf Morphology', *Frontiers in Genetics*, 9. Available at: https://doi.org/10.3389/fgene.2018.00478.

Shepherd, T. and Wynne Griffiths, D. (2006) 'The effects of stress on plant cuticular waxes', *New Phytologist*, 171(3), pp. 469–499. Available at: https://doi.org/10.1111/j.1469-8137.2006.01826.x.

Tilman, D. and Lehman, C. (2001) 'Human-caused environmental change: Impacts on plant diversity and evolution', *Proceedings of the National Academy of Sciences*, 98(10), pp. 5433–5440. Available at: https://doi.org/10.1073/pnas.091093198.
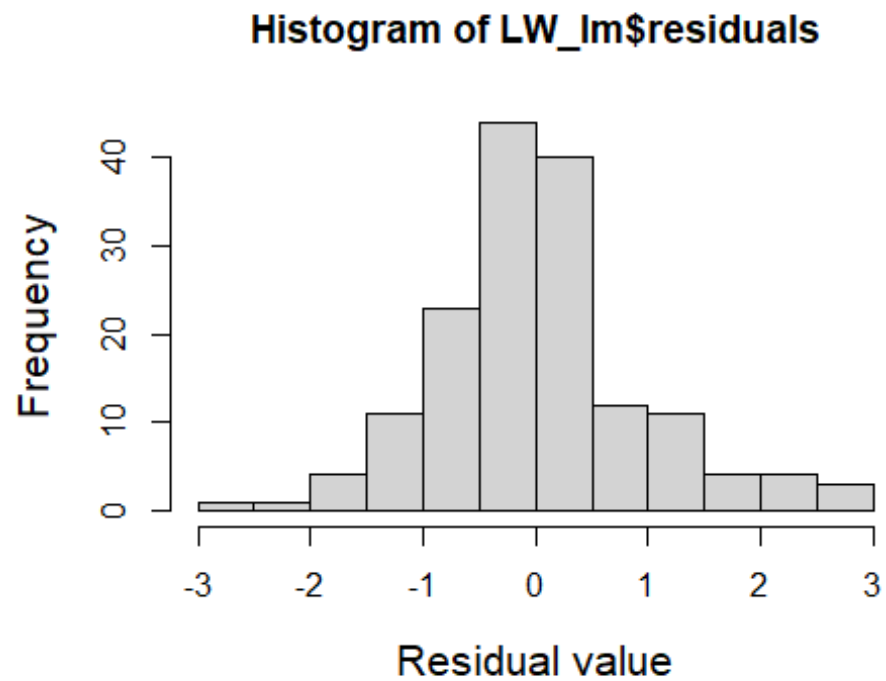
Verdú, M. *et al.* (2003) '"Convergent" traits of mediterranean woody plants belong to pre-mediterranean lineages: EVOLUTIONARY CONVERGENCE IN MEDITERRANEAN PLANTS', *Biological Journal of the Linnean Society*, 78(3), pp. 415–427. Available at: https://doi.org/10.1046/j.1095-8312.2003.00160.x.

Welk, A., Welk, E. and Bruelheide, H. (2014) 'Biotic Interactions Overrule Plant Responses to Climate, Depending on the Species' Biogeography', *PLOS ONE*, 9(10), p. e111023. Available at: https://doi.org/10.1371/journal.pone.0111023.

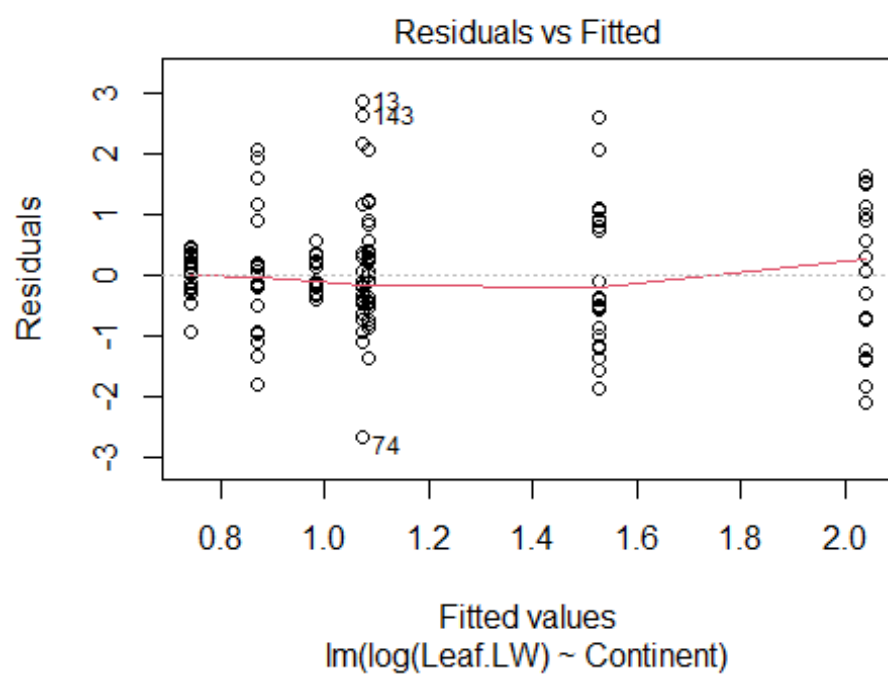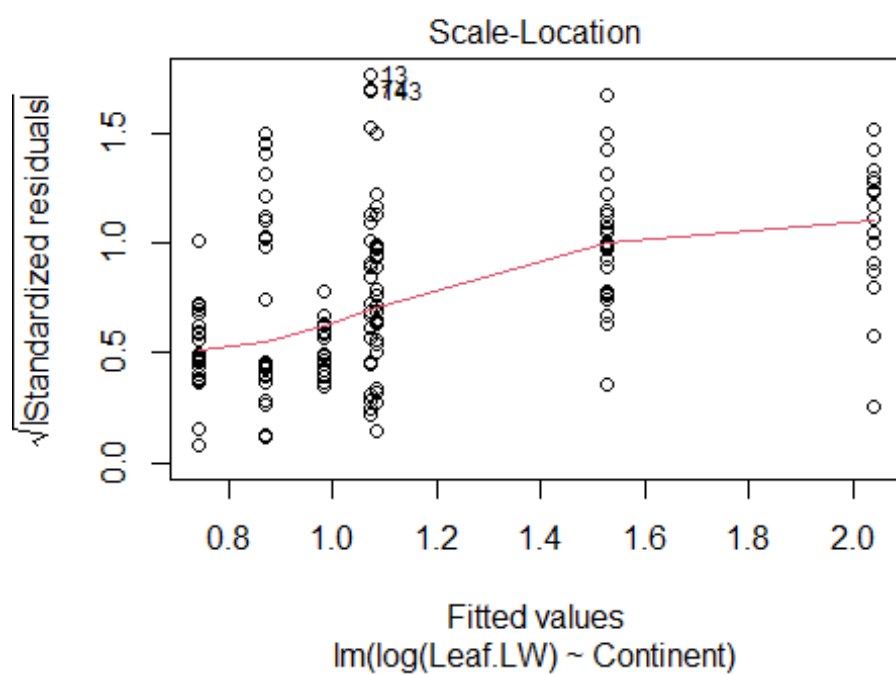Wright, I.J. *et al.* (2017) 'Global climatic drivers of leaf size', *Science*, 357(6354), pp. 917–921. Available at: https://doi.org/10.1126/science.aal4760.

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Supporting Information

Normal Q-Q Plot

Histogram of LW_lm$residuals
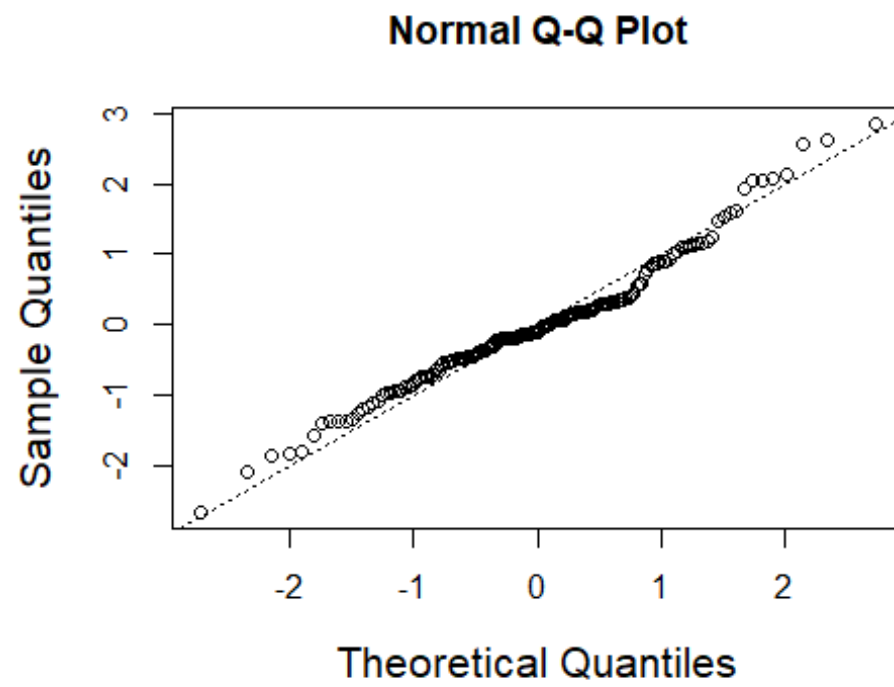

```r
shapiro.test(LW_lm$residuals)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  LW_lm$residuals
## W = 0.97371, p-value = 0.004113
```
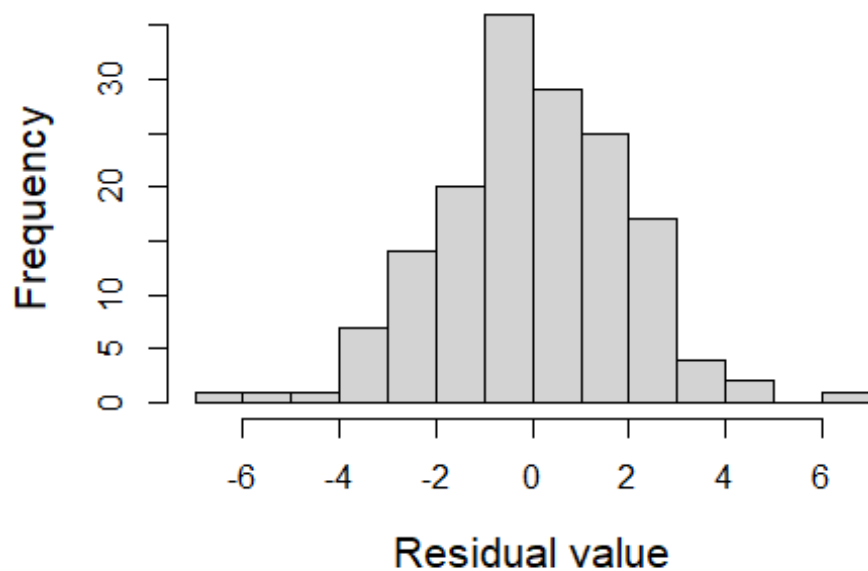
Scale-Location

Fitted values
lm(log(Leaf.LW) ~ Continent)

Residuals vs Fitted

Fitted values
lm(log(Leaf.LW) ~ Continent)

```
bptest(LW_lm)
```

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```
##
##   studentized Breusch-Pagan test
##
## data:  LW_lm
## BP = 22.207, df = 6, p-value = 0.001111
```
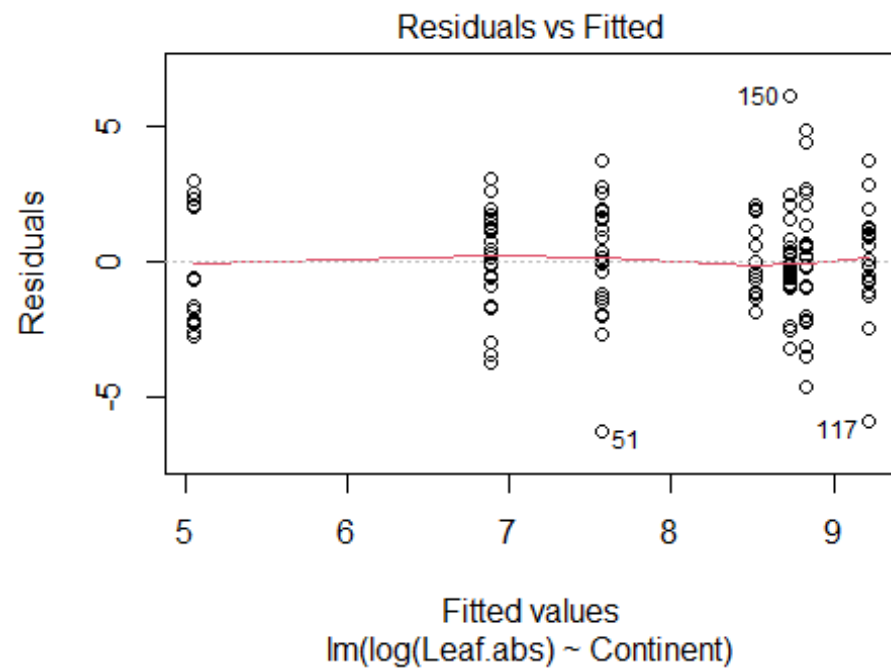
Normal Q-Q Plot
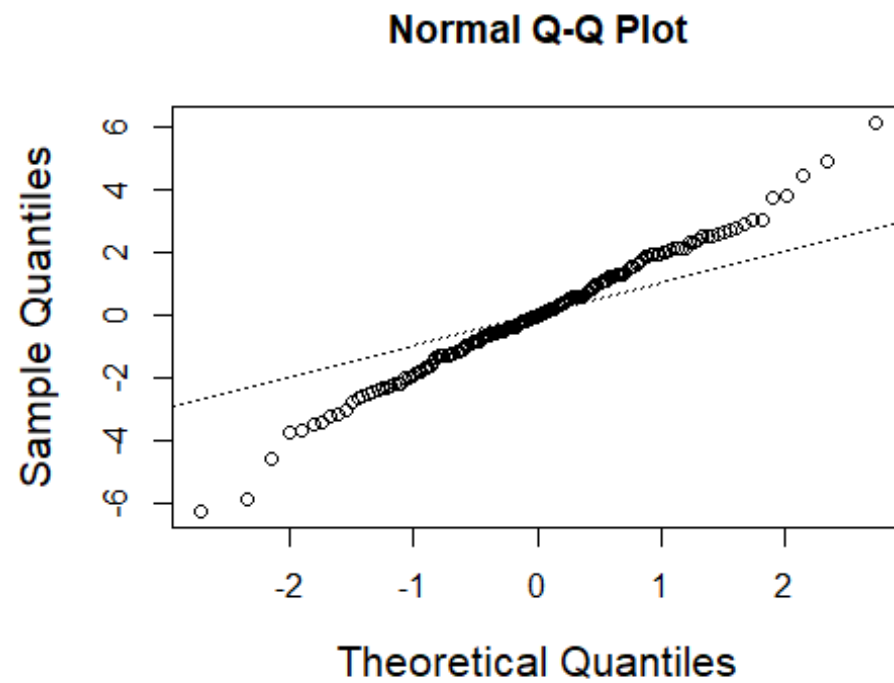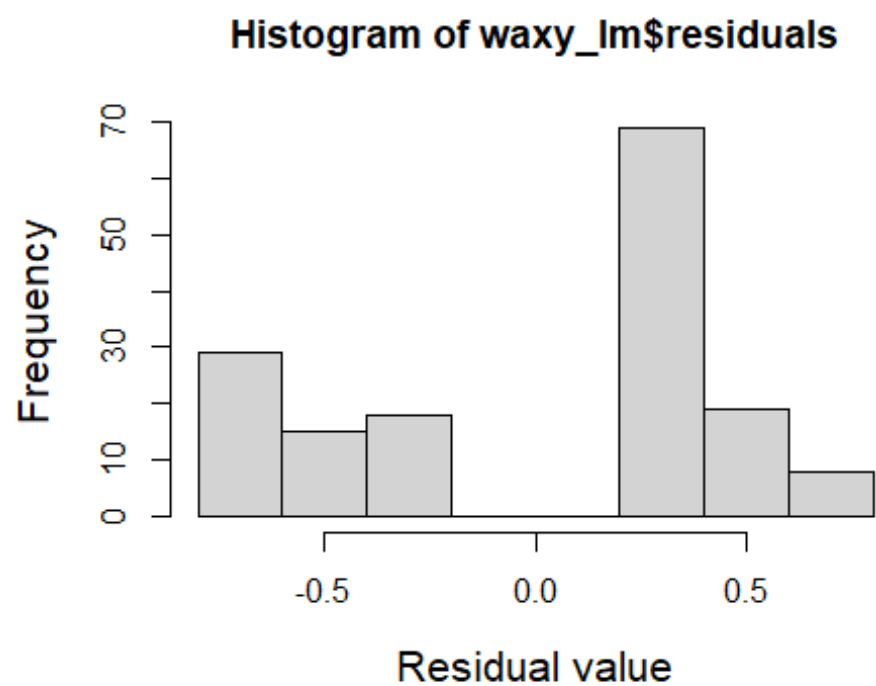
Histogram of abs_lm$residuals
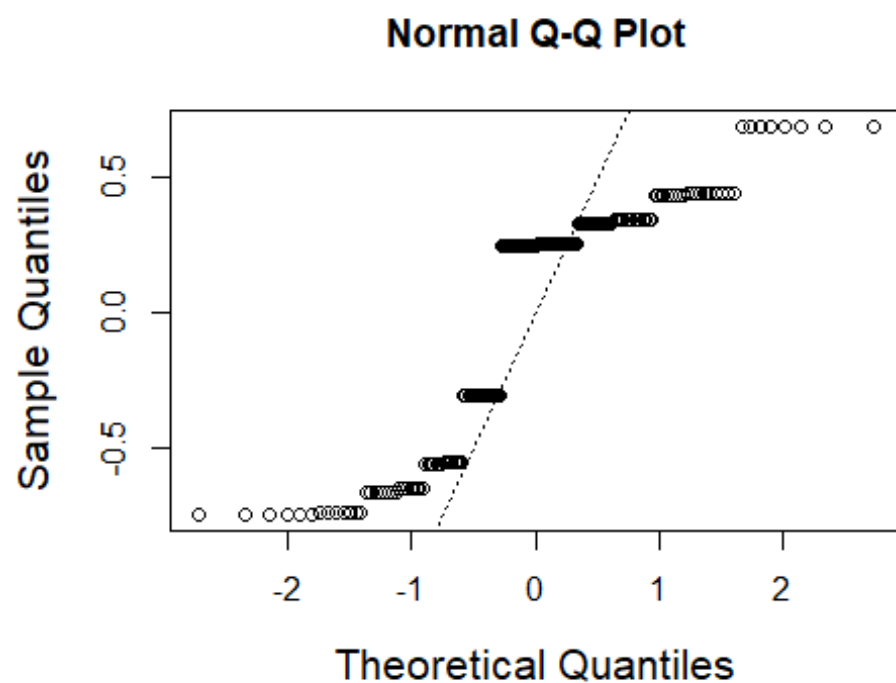


```r
shapiro.test(abs_lm$residuals)

##
##  Shapiro-Wilk normality test
##
## data:  abs_lm$residuals
## W = 0.99209, p-value = 0.5346
```
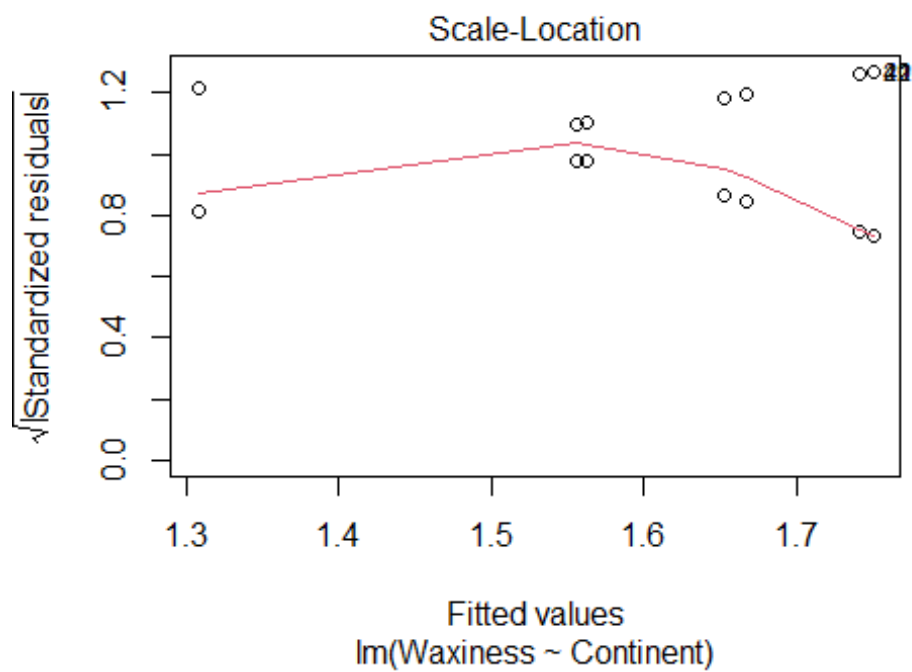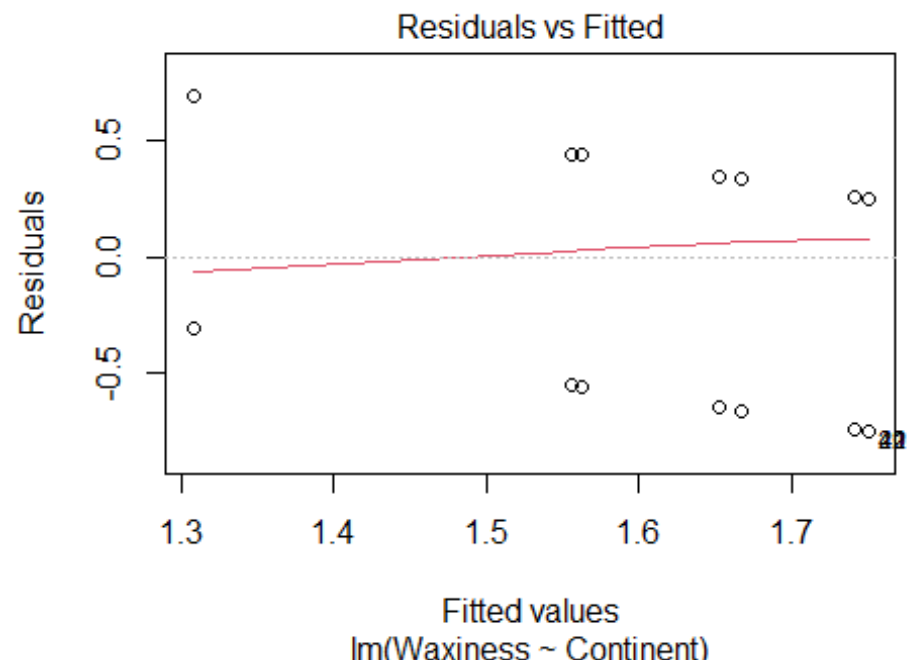
Residuals vs Fitted

lm(log(Leaf.abs) ~ Continent)

Scale-Location

lm(log(Leaf.abs) ~ Continent)

```
bptest(abs_lm)
```

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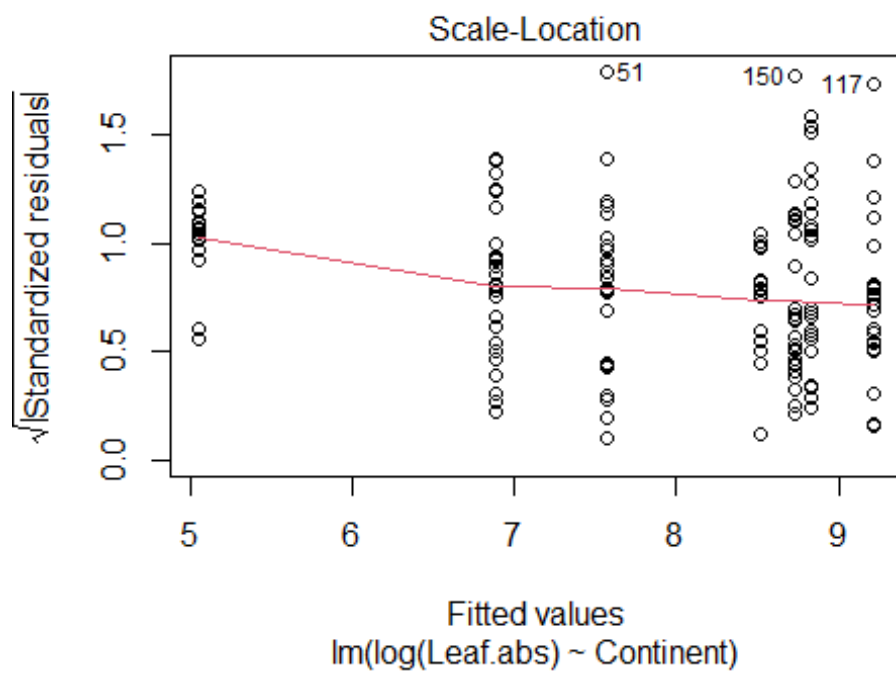
```
## 
##   studentized Breusch-Pagan test
## 
## data:  abs_lm
## BP = 4.1872, df = 6, p-value = 0.6514
```

Normal Q-Q Plot

Histogram of waxy_lm$residuals


```r
shapiro.test(waxy_lm$residuals)
```

```
## 
##  Shapiro-Wilk normality test
## 
## data:  waxy_lm$residuals
## W = 0.83482, p-value = 4.527e-12
```

Residuals vs Fitted

Fitted values
lm(Waxiness ~ Continent)

Scale-Location

Fitted values
lm(Waxiness ~ Continent)

```
bptest(waxy_lm)
```

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```
## 
##  studentized Breusch-Pagan test
## 
## data:  waxy_lm
## BP = 2.5278, df = 6, p-value = 0.8653
```