BATESIAN MIMICRY IN PLANTS: THE
SIGNIFICANCE OF SHARED LEAF SHAPE
BETWEEN *ALSEUOSMIA PUSILLA* AND
PSEUDOWINTERA COLORATA

BY

KARL GRAEME YAGER

A thesis

Submitted to the Victoria University of Wellington

in fulfilment of the requirements for the degree of

Doctor of Philosophy

Victoria University of Wellington

(2018)

Seedlings of *Alseuosmia pusilla* (left) and *Pseudowintera colorata* (right) growing together in a tree fall clearing on the Field Hut Track at Otaki Forks region, Tararua Forest Park, New Zealand.

ABSTRACT

There is an immense amount of variation in leaf shape, size, and colouration, both across and within plant species. Leaf shape and colour, in some instances, can be attributed as a physiological response to particular abiotic stressors. However, leaf shape, size, and colour are used by herbivores to identify sources of palatable foliage for food. It is possible, therefore, that an undefended plant might gain protection from herbivores by matching leaf characteristics of a chemically defended species. The matching of defensive signals by an undefended species in order to dupe a predator is known as Batesian mimicry, and whilst believed to be a relatively common phenomenon amongst animals, it has yet to be proven in plants. The foliage of *Alseuosmia pusilla* (Colenso) A. Cunningham, is strikingly similar to the human eye to that of *Pseudowintera colorata* (Raoul) Dandy, an unrelated sympatric species found in New Zealand. Unlike the foliage of *A. pusilla*, that of *P. colorata* contains a number of secondary metabolites associated with herbivore defence, including a sesquiterpene dialdehyde known as polygodial, a known potent insect antifeedant that imparts a pungent peppery taste when eaten. It has been hypothesised that this similarity evolved under browsing pressure from nine species of large extinct herbivorous birds, collectively known as moa. Whilst moa became extinct soon after the arrival of humans, the large herbivore guild has been effectively replaced by a range of introduced mammalian herbivores including several species of deer, though to what degree remains controversial.

In chapter two, I established a robust spatially explicit morphometric analysis method to test how similar the leaves of *A. pusilla* and *P. colorata* leaves were, and whether leaf shape was a distinctive trait within their shared habitat. Using the Cartesian coordinates of leaf margins as descriptors of leaf shape, I found that *P. colorata* leaves were morphologically distinct from all of the neighbouring species except for those of *A. pusilla*. *A. pusilla* individuals were more similar to neighbouring than to distant *P. colorata*, and 90% of leaf shape variation in the two species varied similarly across an elevational gradient. The data are consistent with Batesian mimicry, wherein the conspicuous characteristic of a defended model is replicated by an undefended mimic across its entire growing range.

In chapter three, I tested how leaf shape variation within, and between, *A. pusilla* and *P. colorata* responded when exposed to high levels of mammalian herbivory. I demonstrated that in a forest population of *P. colorata* and *A. pusilla* exposed to high mammalian herbivory pressure, leaf shape variation is reduced in both focal species, but not in other sympatric

species. This is consistent with Batesian mimicry, wherein increased herbivory pressure selects for a stronger signal in the distinctive characteristic of the defended plant, and through the selection for mimicry, variation in the mimic's phenotype converges on the model's phenotype. Additionally, when alternative palatable food is preferentially targeted, *P. colorata* increased in abundance along with a proportionate increase in *A. pusilla*'s abundance. Invertebrate herbivory was estimated to be similar on both species at both sites.

In chapter four, I tested the hypothesis that *A. pusilla* is a Batesian mimic of *P. colorata* using farmed red deer (*Cervus elaphus scoticus*) in feeding trials. The deer found *A. pusilla* more palatable than *P. colorata*, and after eating a *P. colorata* individual, they became reluctant to eat another plant. Although the two plants differ significantly in volatile organic compound emissions, deer were equally likely to first eat an *A. pusilla* as they were a *P. colorata*, therefore were unable to use olfactory cues, or visually differentiate between the two species. As the relative abundance of *P. colorata* increased, herbivory damage was lower, both in the defended *P. colorata* and in the undefended *A. pusilla*. This study provides the first unequivocal proof of defensive Batesian mimicry in plants.

In chapter five, using humans as surrogate herbivores, I tested how leaf shape and colour can be used as cues or signals by herbivores when foraging for food under different conditions. Subjects found leaf size a distracting characteristic, foraging more effectively when *A. pusilla* and *P. colorata* individuals were most similar in 94% of their shared shape variation. The trait of leaf colour, whilst unreliable by itself, acted to potentiate the trait of leaf shape, as a signal or cue. Fast feedback on species palatability improved accuracy in identifying *A. pusilla*, but neither fast nor slow feedback improved discriminability of *P. colorata*. *A. pusilla* leaves were harder to discriminate when presented on a “disruptive” backdrop. My results demonstrate that leaf shape can act as a signal or cue. These results indicate why further research into plant-herbivore communication is important and that it could provide powerful insights into the functional significance of leaf morphology.

This thesis provides a significant contribution to our understanding of how leaves function as signals or cues to herbivores in three ways: (i) it provides the first detailed and powerful quantitative evidence of leaf shape matching between two species, and demonstrates the importance of using a spatially explicit morphometric method when investigating leaf shape; (ii) it is the first to unequivocally prove defensive Batesian mimicry in plants; and (iii) it demonstrates that leaf traits can act as signals or cues.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the ongoing support of my primary supervisor, Kevin Gould, whose patience, encouragement, and guidance has been endless. Without your support and understanding I would not have been able to contemplate finishing this thesis. I have also benefited hugely from the vast breadth of scientific and academic knowledge of Martin Schaefer. Martin's considered and thoughtful feedback and suggestions has been invaluable. A big thank you is required to Bart Cox, Mikey Wilcox, Timo Brockmeyer, Loïc Cizabuiroz, Catherine Samson, Nigel Perry and John van Klink for their field and technical assistance. Thank you to the Department of Conservation and Greater Wellington Regional Council for field work permits. I would also like to thank members of the Gould and Davy labs; Luke Cooney, Ignatius Menzies, Kate Calcott, Gagandeep Jain, Maheshini Mawalagedera, Emma Gibbin, Shaun Wilkinson, Katie Hillyer and Anne Wietheger, whom I shared a stimulating and exciting work environment with. Thank you also to Patricia Stein who has been an administrative saviour. Lastly, a special thanks to Jon Redmond for the use of his deer farm, carrying my plants back and forth to the study site and sharing many cups of coffee.

This research was funded by a Victoria University of Wellington Doctoral Scholarship.

On a personal note, to my wife Danielle, you have endured a lot. This has not been plain sailing. Whilst life events have stood in our way, you have been a vessel of love and support. To Noah, thank you for waiting patiently to share your love and joy with me whilst I have been mentally absent. I look forward to being present more often for you, especially with the arrival of your baby brother(s) and/or sister(s).

TABLE OF CONTENTS

FRONTISPIECE.....	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	xii
LIST OF TABLES.....	xviii
1. Chapter 1: General Introduction.....	1
1.1 Plant leaves.....	1
1.1.1 Functional significance of leaf pigments	1
1.1.2 Functional significance of leaf morphology	2
1.2 Plant herbivore interactions	4
1.2.1 Plant defences	4
1.2.2 Herbivore foraging	6
1.2.3 Impact of invertebrate and vertebrate herbivory	7
1.2.4 Foraging theory	7
1.3 Learning about food	8
1.3.1 Predator-prey learning	8
1.3.2 Use of visual signals or cues to avoid unpalatable prey	8
1.3.3 Use of visual warning signals by prey	9
1.3.4 Non-aposematic visual signals	10
1.3.5 Predator attacking strategy	10
1.4 Dishonest defensive signals	11
1.4.1 Batesian mimicry	11
1.4.2 Imperfect mimicry	12
1.4.3 Imperfect mimicry hypotheses with empirical support for Batesian mimicry	12
1.4.4 Imperfect mimicry hypotheses with no empirical support for Batesian mimicry	15
1.4.5 Multiple hypotheses in a single system	17
1.5 Leaf shape and colour influences consumption and performance	17
1.6 Batesian mimicry in plants	18

1.6.1	Postulated cases of defensive plant-plant mimicry – a review	18
1.6.2	Human-induced mimicry in plants	19
1.7	Gap in knowledge	20
1.8	<i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i> are model species to test for Batesian mimicry	20
1.9	Aims of the thesis	21
1.9.1	Specific objectives	21
2.	Chapter 2: The significance of shared leaf shape in <i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i>	23
2.1	Abstract	23
2.2	Introduction	23
2.3	Material and Methods	26
2.3.1	Plant Material	26
2.3.2	Leaf Shape Capturing	27
2.3.3	Leaf Colour	27
2.3.4	Statistical Analysis	28
2.4	Results	29
2.4.1	Leaf Morphology	29
2.4.2	Shape Analysis	30
2.4.3	Shape Discrimination	32
2.4.4	Elevational variation in leaf shape	33
2.4.5	Shape comparison among neighbours	34
2.5	Discussion	36
3.	Chapter 3: Introduced mammalian herbivores select for increased distinctiveness within <i>Pseudowintera colorata</i> and its purported mimic <i>Alseuosmia pusilla</i>	39
3.1	Abstract	39
3.2	Introduction	40
3.3	Material and Methods	44
3.3.1	Study Sites	44
3.3.2	Plant Material	45
3.3.3	Herbivory, abundance and neighbour proximity estimation	45
3.3.4	Leaf Shape Capturing	46
3.3.5	Statistical Analysis	46
3.4	Results	47

3.4.1	Leaf Morphology	47
3.4.2	Shape Analysis	48
3.4.3	Leaf shape variation between sites	50
3.4.4	Elevational variation in leaf shape within sites	50
3.4.5	Shape comparison among neighbours	53
3.4.6	Shape Discrimination	55
3.4.7	Species abundance between sites	56
3.5	Discussion	58
4.	Chapter 4: Defensive Batesian mimicry deceives an introduced herbivore	63
4.1	Abstract	63
4.2	Introduction	64
4.3	Material and Methods	67
4.3.1	Plant Material	67
4.3.2	Volatile Assay	67
4.3.3	SPME Absorption	68
4.3.4	GC-MS Procedure	68
4.3.5	Animal Subjects	68
4.3.6	Camera Equipment	69
4.3.7	Experimental Design	69
4.3.8	Statistical Analysis	72
4.4	Results	72
4.4.1	VOC Emissions	72
4.4.2	Feeding trials over 24 hours	74
4.4.3	Feeding trials during daylight	77
4.5	Discussion	81
5.	Chapter 5: The use of leaf traits to discriminate between a purported mimic and its model	86
5.1	Abstract	86
5.2	Introduction	86
5.3	Material and Methods	91
5.3.1	Plant Material	91
5.3.2	Leaf Shape Capturing and Analysis	91
5.3.3	Survey Setup	91
5.3.4	Predators	94

5.3.5	Prey	95
5.3.6	Standard Conditions	96
5.3.7	Testing Background	97
5.3.8	Subjects' use of traits	98
5.3.9	Statistical Analysis	98
5.4	Results	100
5.4.1	How the variation in leaf shape between mimics and models affects subjects' identification abilities	102
5.4.2	How leaf traits affect subjects' identification abilities	104
5.4.3	How the amount of variation in leaf shape between mimics and models affects subjects' use of leaf trait characteristics for identification ...	105
5.4.4	How did feedback affect subjects' identification abilities?	107
5.4.5	How does the ratio of models to mimics affect subjects' identification ability?	107
5.4.6	How does the background affect subjects' identification ability?	109
5.5	Discussion	110
6.	General Discussion	117
6.1	Introduction	117
6.2	Novel discoveries from this thesis	117
6.3	The significance of establishing <i>Alseuosmia pusilla</i> as a Batesian mimic of <i>Pseudowintera colorata</i>	118
6.3.1	Leaf shape similarity across the entire shared range	119
6.3.2	Leaf shape variability when exposed to high and low herbivory pressure	120
6.3.3	Testing the Batesian mimicry hypothesis in plants	120
6.4	Leaf traits as cues and signals, and the function of feedback and backdrop	121
6.4.1	Multidimensional signals in mimicry	122
6.4.2	Multidimensional signals in leaves	122
6.4.3	Feedback and backdrop	122
6.4.4	Plant-herbivore communication	123
6.5	Conclusions	123
APPENDICES.....		127
A	Additional information for Chapter 2	127
B	Additional information for Chapter 3	129

C	Additional information for Chapter 4	131
D	Additional information for Chapter 5	132
REFERENCES		135

LIST OF FIGURES

- Fig. 1.1. The variation in leaf shape in a small selection of leaves as seen in 100 m² of forest. (A) *Knightia excels*, (B) *Coprosma grandifolia*, (C) *Myrsine salicina*, (D) *Griselinia littoralis*, (E) *Pseudowintera colorata*, (F) *Raukaua simplex*, (G) *C. foetidissima*, (H) *Lophozonia menziesii*, (I) *C. colensoi*, (J) *Lophomyrtus bullata*, (K) *Prumnopitys ferruginea*, (L) *Alseuosmia pusilla* 3
- Fig. 2.1. Leaf morphology of *Pseudowintera colorata* and *Alseuosmia pusilla*. (A) *P. colorata* (P.c) and *A. pusilla* (A.p) seedlings growing together under forest canopy (top), and in the open due to tree fall (bottom). (B) Selected examples to show the range of *A. pusilla*, and (C) *P. colorata* leaves across the elevational gradient 26
- Fig. 2.2. Reflectance spectra of leaves. Proportions of reflected light from (A) green, and (B) red portions of *Pseudowintera colorata* (dashed grey line) and *Alseuosmia pusilla* (solid black line) lamina..... 30
- Fig. 2.3. Principal components analysis (PCA) of leaf shape variation in *Pseudowintera colorata* and *Alseuosmia pusilla*. PCA reduced 98.1% of leaf shape variation between the two species into four principal components (PC1-4). For each PC, proportionate frequencies of *P. colorata* (grey bars) and *A. pusilla* (black bars) leaves are given for up to 3 standard deviations (S.D.) of the mean leaf shape (0). Leaf silhouettes show typical shapes, proportionately scaled, represented by each 1 S.D. on each PC. (A) PC1, accounted for 89.6% of total leaf shape variation; (B) PC2, 4.1%; (C) PC3, 3.4%; (D) PC4, 1% 31
- Fig. 2.4. Leaf shape of *Alseuosmia pusilla* and *Pseudowintera colorata* in relation to elevation. Leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, as illustrated in Fig. 3. *A. pusilla* (crosses) and *P. colorata* (circles). Each point represents one leaf from an individual plant. The y-axes extend ± 5 S.D. along each PC from the mean leaf shape, which is represented by 0. Linear regressions show elevational trends in leaf shape variation explained by each PC for *A. pusilla* (black line) and *P. colorata* (grey line). Leaf silhouettes are proportionately scaled and show typical shapes represented by ± 3 S.D. and the mean leaf shape (0) 34
- Fig. 2.5. Comparison of individual *Alseuosmia pusilla* principal component (PC) values to the neighbouring *Pseudowintera colorata* population. Log-likelihood scores for

similarity in leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, comparing *A. pusilla* individuals to the surrounding *P. colorata* over distance 35

Fig. 2.6. Comparison of individual *Pseudowintera colorata* principal component (PC) values to the neighbouring *Alseuosmia pusilla* population. Log-likelihood scores for similarity in leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, comparing *P. colorata* individuals to the surrounding *A. pusilla* over distance..... 36

Fig. 3.1. Location of field sites within the south-western portion of the Tararua Forest Park, near Wellington, New Zealand. (A) Kapakapanui field site; “High Herbivore”, and (B) Otaki Forks field site; “Low Herbivore”. Project Kākā pest control area denoted by red shading..... 43

Fig. 3.2 Changes in estimated population densities of ungulates (deer and goat) (A), and possums (B), between Otaki Forks; “Low Herbivore” field site (grey line), and Kapakapanui; “High Herbivore” field site (black line)..... 44

Fig. 3.3. Selected examples to show the range of leaf morphology across the elevational gradient of (A) *Alseuosmia pusilla* and (B) *Pseudowintera colorata* from Kapakapanui, “High Herbivore” site, and (C) *A. pusilla*, and (D) *P. colorata* from Otaki Forks, “Low Herbivore” site 47

Fig. 3.4. Principal components analysis (PCA) of leaf shape variation in *Pseudowintera colorata* and *Alseuosmia pusilla*. PCA reduced 97.7% of leaf shape variation between the two species into four principal components (PC1-4). For each PC, proportionate frequencies of *P. colorata* (High Herbivore = grey horizontal lined bars, Low Herbivore = black hash bars) and *A. pusilla* (High Herbivore = solid grey bars, Low Herbivore = solid black bars) leaves are given for up to 3 standard deviations (S.D.) of the mean leaf shape (0). Leaf silhouettes show typical shapes, proportionately scaled, represented by each 1 S.D. on each PC. (A) PC1, accounted for 87.6% of total leaf shape variation; (B) PC2, 4.7%; (C) PC3, 4.3%; (D) PC4, 1.1%..... 49

Fig. 3.5. Leaf shape of *Alseuosmia pusilla* and *Pseudowintera colorata* in relation to elevation. Leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, as illustrated in Fig. 3.4. *A. pusilla* (High Herbivore = grey circles, Low Herbivore =

black circles) and *P. colorata* (High Herbivore = grey crosses, Low Herbivore = black crosses). Each point represents one leaf from an individual plant. The y-axes extend ± 7 S.D. along each PC from the mean leaf shape, which is represented by 0. Linear regressions show elevational trends in leaf shape variation explained by each PC for *A. pusilla* (High Herbivore = grey line, Low Herbivore = black line) and *P. colorata* (High Herbivore = dashed grey line, Low Herbivore = black dashed line). Leaf silhouettes are proportionately scaled and show typical shapes represented by ± 3 S.D. and the mean leaf shape (0)..... 52

Fig. 3.6. Comparison of individual *Alseuosmia pusilla* principal component (PC) values to the neighbouring *Pseudowintera colorata* population at the High Herbivore site. The first four PC's account for 96.7% of the total leaf shape variation. Log-likelihood scores for similarity in leaf shape represented by (A) PC1 (82.5%), (B) PC2 (7.4%), (C) PC3 (5.4%), and (D) PC4 (1.4%), comparing *A. pusilla* individuals to the surrounding *P. colorata* over distance..... 54

Fig. 3.7. Comparison of individual *Pseudowintera colorata* principal component (PC) values to the neighbouring *Alseuosmia pusilla* population at the High Herbivore site. The first four PC's account for 96.7% of the total leaf shape variation. Log-likelihood scores for similarity in leaf shape represented by (A) PC1 (82.5%), (B) PC2 (7.4%), (C) PC3 (5.4%), and (D) PC4 (1.4%), comparing *P. colorata* individuals to the surrounding *A. pusilla* over distance 55

Fig. 3.8. Box-and-whiskers plots of the proximity to *Pseudowintera colorata* individuals of eight tree and shrub species growing at Otaki Forks “Low Herbivore site” (LH) (light boxes) and Kapakapanui “High Herbivore site”(HH) (grey boxes) including *Alseuosmia pusilla* (*A.p*). Tree and shrub species common to both sites and sympatric to *A. pusilla* and *P. colorata*..... 57

Fig. 3.9. Visually estimated area (%) of leaf lamina removed by herbivory for *Pseudowintera colorata* (*P.c*) and *Alseuosmia pusilla* (*A.p*) at Otaki Forks “Low Herbivore site” (LH) (dark bars), and Kapakapanui “High Herbivore site” (HH) (light bars) from plots that contained different proportions of *A. pusilla* to *P. colorata* 58

Fig. 4.1. A still capture from a trial video showing the 2 x 3 layout of the plants, the planter bags, and how they were pegged into the ground. To identify deer individuals, the

yellow ear tags displayed a unique number, and other identifying features, such as body markings and/or the position of additional ear tags, could be used	71
Fig. 4.2. VOC emission profiles from leaves of <i>Alseuosmia pusilla</i> (dark bars) and <i>Pseudowintera colorata</i> (light bars).....	73
Fig. 4.3. Box-and-whiskers plots of the amount of foliage consumed by deer from each trail over (A) 24 hours for <i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i> plants, and (B) 10 hours for <i>A. pusilla</i> and <i>P. colorata</i> plants	75
Fig. 4.4. The amount of foliage remaining on <i>Alseuosmia pusilla</i> after being exposed to deer browsing for 10 hours across different proportions of <i>A. pusilla</i> to <i>Pseudowintera colorata</i> . Each point represents one <i>A. pusilla</i> , there may be multiple individuals at each point.....	78
Fig. 4.5. Box-and-whiskers plots for the time taken (in seconds) for an individual deer to eat another plant (either an <i>Alseuosmia pusilla</i> or <i>Pseudowintera colorata</i>), after its first contact (eating) in the trial was with either an <i>A. pusilla</i> or <i>P. colorata</i> individual.....	81
Fig. 5.1. Assignment of the 14 surveys and the variables that were tested. There were two survey types issued, “Plain Background Survey” and “Disruptive Background Survey”. Survey assignment was randomly allocated below the “Background type” level by Qualtrics. The term “dimensional” describes the number of traits that require different cognitive attention.....	92
Fig. 5.2. An example of the same screen presented to subjects across the three trait levels being tested within the <i>Similar</i> survey with no “disruptive” background image; (A) leaf colour only, (B) leaf shape only, and (C) leaf colour and shape. Location of individual leaves within each screen were the same between each trait being tested, but the order in which the screens were presented to subjects was random. The type of survey a subject was given was randomly allocated by the survey programme, Qualtrics, including whether feedback on subjects choices was instant or delayed.....	93
Fig. 5.3. The screen used for the leaf colour and shape and leaf colour only <i>Similar</i> grouped surveys shown to subjects before they started identifying mimics. Subjects who were to be presented the survey testing the trait leaf shape only were presented a	

similar screen but leaves were of a uniform colour as depicted in Fig. 5.2B. Subjects were allowed to spend as long as they wanted to “learn” differences between the two species. Images of leaves that depicted the traits leaf colour and shape were replaced with images of leaves with either the trait leaf shape and colour or leaf colour only for participants given the corresponding versions of the survey 95

Fig. 5.4. An example of a screen from the *Similar* survey with leaves on a “disruptive” background image 98

Fig. 5.5. The proportion of mimics (dark bars) and models (light bars) subjects selected when mimics and models were dissimilar (top row) and similar (bottom row) in leaf shape (*Dissimilar* and *Similar* leaf groupings) within the three trait types (columns) tested at the two types of feedback..... 102

Fig. 5.6. Subjects ability to differentiate between mimics and models as quantified by the signal detection theory measure of sensitivity statistic, A , between groupings of mimics and models that were dissimilar (top row) and similar (bottom row) in leaf shape (*Dissimilar* and *Similar* leaf groupings) within the three trait types (columns) tested under delayed feedback (dark bars) and instant feedback (light bars). A higher value equates to greater sensitivity in subjects 103

Fig. 5.7. The leaf trait characteristics subjects used to identify mimics and models. Traits used affected their final score (left vertical axis), however the proportion of subjects that used each trait varied (right vertical axis). (A) Leaf shape only, within the *Dissimilar* leaf group (dark bars) and the *Similar* leaf group (light bars), and (B) when leaf shape and colour were combined, within the *Dissimilar* leaf group (dark bars) and the *Similar* leaf group (light bars). A perfect score where every mimic was selected and every model was avoided would equal 99. Lines indicate the proportion of participants who identified using any given leaf trait characteristic (*Dissimilar*: dark bars, *Similar*: light bars) 106

Fig. 5.8. The proportion of mimics (dark bars) and models (light bars) subjects selected when mimics and models were (A) dissimilar in leaf shape (*Dissimilar* leaf grouping), and (B) similar in leaf shape (*Similar* leaf grouping) across the three ratios (3:1, 1:1, 1:3) of mimics to models within the three trait types tested (leaf shape and colour, leaf shape only, and colour only). The first number in the ratio

mix equates to the number of mimics, and the second number equates to
models 108

Fig. 5.9. Subjects' ability to differentiate between mimics and models as quantified by the signal detection theory measure of sensitivity statistic, A , from between groupings of mimics and models that were similar in leaf shape (*Similar* leaf grouping), across the three ratios of mimics to models, within the three leaf trait types tested. A higher value equates to greater sensitivity in subjects..... 109

Fig. 5.10. (A) The proportion of mimics (dark bars) and models (light bars) subjects selected when leaves were presented on a plain white background and a “disruptive” background. Mimics and models were dissimilar in leaf shape (*Dissimilar* leaf grouping) and similar in leaf shape (*Similar* leaf grouping). (B) Subjects ability to differentiate between mimics and models as quantified by the signal detection statistic measure of sensitivity statistic, A , between groupings of mimics and models that are presented on a “disruptive” background (dark bar) and on a plain white background (light bar). 110

LIST OF TABLES

Table 2.1. Percentage of correct identification of species by cross validated discriminant analysis using the leaf margin landmarks	32
Table 3.1. Levene's test of equal variances between the High Herbivore (HH) and Low Herbivore (LH) sites for the four principal components (PC) representing 97.7% of the total shape variation between <i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i> , and 96.3% between seven sympatric tree and shrub species common to both sites (PC1: 77.8%, PC2: 8.2%, PC3, 6.9%, PC4: 3.4%)	50
Table 3.2. Linear regressions for each principal component (PC) as a function of elevation compared for each species by ANCOVA. (A) The two species response to elevation is similar if the slopes are parallel ($p > 0.05$) on the (B) combined linear regression of species. (C) The intercepts between the two species do not differ if $p > 0.05$, therefore the magnitude of response over elevation is closer between species than when intercepts are different ($p < 0.05$)	51
Table 3.3. Percentage of correct identification of <i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i> by cross validated discriminant analysis using the leaf margin landmarks at the High and Low Herbivore sites.....	56
Table 4.1. VOC compounds found in <i>Alseuosmia pusilla</i> leaves that are either absent, or are found in concentrations that are less than (–), similar to (=), or greater than (+) in <i>Pseudowintera colorata</i> leaves. The level of difference in concentrations is a measure of “times greater” between each species mean concentrations for each compound.....	74
Table 4.2. Model formulae, coefficients, and standard errors, test statistics, and significance values from log transformed Poisson generalised linear mixed models for the amount of foliage browsed from <i>Alseuosmia pusilla</i> and <i>Pseudowintera colorata</i> as the proportion of <i>P. colorata</i> to <i>A. pusilla</i> increased over (A) 24 hour trials, and (B) 10 hour trials	76
Table 4.3. Model formulae, coefficients, and standard errors, test statistics, and significance values from a log transformed Poisson generalised linear model for the amount of	

foliage browsed from <i>Alseuosmia pusilla</i> as the proportion of <i>P. colorata</i> to <i>A. pusilla</i> increased over the 10 hour trials	77
Table 4.4. Species known to be palatable to red deer, <i>Hoheria sexstylosa</i> and <i>Griselinia littoralis</i> , were fed to deer at four different proportions to <i>P. colorata</i> (n = 1 trial at each proportion). The proportion of foliage removed on each plant for both the palatable species and <i>P. colorata</i> was recorded	79
Table 4.5. Model formulae, coefficients, and standard errors, test statistics, and significance values from a log transformed Poisson generalised linear model for the amount of foliage browsed from the palatable species (A) <i>Hoheria sexstylosa</i> , and (B) <i>Griselinia littoralis</i> , as the proportion of <i>P. colorata</i> to palatable species increased over the 10 hour trials	79
Table 4.6. Model formulae, coefficients, and standard errors, test statistics, and significance values from the log transformed Poisson generalised linear mixed model for the time taken to eat for an individual deer to eat another plant (either an <i>Alseuosmia pusilla</i> or <i>Pseudowintera colorata</i>), after its first contact (eating) in the trial was with either an <i>A. pusilla</i> or <i>P. colorata</i> individual during a 10 hour trial	80
Table 5.1 Statistically significant MANOVA results between leaf groupings and within leaf groupings for the proportion of mimics and models selected by subjects, and subjects' level of sensitivity as measured by the SDT measure, <i>A</i>	101

Chapter 1: General Introduction

It is estimated that there may be as many as 500,000 species of vascular and non-vascular plants (Corlett 2016), and this diversity has strong bottom-up effects on biotic interactions and the diversity of other terrestrial organisms (Scherber et al. 2010, Isbell et al. 2011). Just as the diversity of plant species is immense (Christenhusz and Byng, 2016), there is also huge diversity in plant morphology, both in the overall architecture (Barthélémy and Caraglio, 2007) and in the constituent organs (Nicotra et al. 2011), as well as substantial variation in leaf colour (Lev-Yadun et al. 2004, Schaefer and Rolshausen, 2005). Each plant species has a range of ecological interactions between them and their environment, and within and between species across many taxa.

The functional significance of morphological and colour variation in leaves is not always apparent. The genetic controls of leaf shape are becoming better understood (Tsukaya 2005, Klingenberg et al. 2012, Dkhar and Pareek 2014), and while the physiological benefits of different leaf shapes are not as well studied (Kidner and Umbreen 2010, Nicotra et al. 2011), even less understood is the influence herbivores may have on leaf morphology and colouration.

1.1 Plant leaves

Leaves have evolved independently within several lineages of vascular plants and are primarily photosynthetic organs (Nicotra et al. 2011). While their main function is to convert light energy into chemical energy required for plant growth and reproduction, there exists an enormous amount of variation in leaf colour, shape and size, both within and between species (Lev-Yadun et al. 2004, Nicotra et al. 2011).

1.1.1 Functional significance of leaf pigments

Leaves are generally green because the *a* and *b* chlorophyll pigments of photosynthesis strongly absorb blue and red light (Nishio 2000), and only weakly absorb green, enabling high photosynthetic efficiency under highly variable light conditions (Nishio 2000, Terashima et al. 2009). Leaves appear green to humans because of the absorption properties of chlorophyll pigments. There are often two other pigments present in leaves: carotenoids, which primarily absorb blue light, appearing yellow through to orange, and anthocyanins, which absorb light in the yellow-green wavelengths, appearing red. Carotenoids can aid photosynthetic activity through increased light absorption and photoprotection (Siefermann-

Harms 1985). However, the adaptive significance of anthocyanin pigments in leaves has been the subject of debate for over a century (Gould 2010).

Anthocyanic leaves are produced throughout almost all orders of the plant kingdom and across all terrestrial habitats (Lee 2002). Where and when these red pigments occur in leaves can vary within and between species. Anthocyanin pigments may only be present during a particular developmental phase of a leaf (Coley and Aide 1989, Gould 2004, Ougham et al. 2005, Archetti 2009a), be associated with a particular climatic season (Hughes 2011), or be present in leaves across their lifespan (Gould et al. 2000). Anthocyanic red pigmentation may occur uniformly across a leaf or be localised to specific areas of a leaf (Lee 2007, Cooney et al. 2012, Hughes and Lev-Yadun 2015).

The role of anthocyanins in leaves can be attributed to photoprotection (Neill and Gould 2003, Hughes et al. 2005, Gould et al. 2010), but they may also have a role in reducing herbivory in plants, either helping with camouflaging leaves (Fadzly et al. 2009, Klooster et al. 2009, Fadzly and Burns 2010), or as a signal of the level of invested chemical defence (Cooney et al. 2012, Hughes and Lev-Yadun 2015), and/or reduced nutrient content (Hamilton and Brown 2001, Archetti and Brown 2004, Archetti 2009b).

1.1.2 Functional significance of leaf morphology

The morphology of leaves is diverse (Fig. 1.1), especially amongst the angiosperms (Nicotra et al. 2011). A leaf may be simple, with a single leaf lamina, or compound, with several leaflets. There may be several components making up a leaf each with their own leaflets, and the leaflets themselves may be simple or compound. Leaves may be long and narrow, or short and wide. Leaf tips and bases can vary in angles, and leaf margins may be smooth, lobed, or toothed. Plant leaf morphology is not static. That is, many plant species exhibit morphological plasticity in response to ontogenetic, abiotic, competitive, and predatory pressures. These traits often fluctuate predictably across environmental gradients, suggesting that phenotypic plasticity confers a selective advantage to a plant in its natural habitat (Hovenden and Vander Schoor 2006, Royer et al. 2009).

Fig. 1.1. The variation in leaf shape in a small selection of leaves as seen in 100 m² of forest. (A) *Knightia excelsa*, (B) *Coprosma grandifolia*, (C) *Myrsine salicina*, (D) *Griselinia littoralis*, (E) *Pseudowintera colorata*, (F) *Raukaua simplex*, (G) *Coprosma foetidissima*, (H) *Lophozonia menziesii*, (I) *Coprosma colensoi*, (J) *Lophomyrtus bullata*, (K) *Prumnopitys ferruginea*, (L) *Alseuosmia pusilla*. Location of survey – Tararua Forest Park, New Zealand (S40 53.30, E175 14.22 to S40 54.46, E175 15.37). Scale bar = 20 mm.

Leaf shape, for example, can assist in temperature and hydraulic regulation. Lobed or margin dissected leaves can cool more efficiently (Vogel 1968, Gurevitch and Schuepp 1990), and are also more efficient in dry conditions (Thoday 1931). However, the extent of lobing may in itself be determined by hydraulic limitations (Zwieniecki et al. 2004, Boyce 2009). Under salt stress leaves become narrower (Anne et al. 1998, Sinclair and Hoffmann 2003). Leaf teeth are globally associated with plant communities from regions of low mean annual temperatures (Anderson et al. 2005, Peppe et al. 2011), where they may promote faster early leaf development (Royer and Wilf 2006). Leaf size decreases with increasing altitude,

decreasing rainfall, and decreasing soil nutrient content (McDonald et al. 2003, Nicotra et al. 2011, Milla and Reich 2011). Smaller sized leaves are also better adapted to dry or hot environments (Scoffoni et al. 2011). Within these broad trends, however, there is often considerable intraspecific variation in leaf shape even in the same environment (McDonald et al. 2003, Nicotra et al. 2011). Under a variety of conditions, a specific shape of leaf is not clearly attributable to a particular condition. Yet change in leaf shape within particular species has been shown to be advantageous, for example, in light interception (Horn 1971, Tsukaya 2005), for minimising wind damage (Anten et al. 2010), during drought, for nutrient deficiencies (Cunningham et al. 1999, Leigh et al. 2011), or to deter predation by herbivores (Niemelä and Tuomi 1987, Brown and Lawton 1991, Dell'aglio et al. 2016). The possible functional significance of such variation is usually far from clear.

1.2 *Plant herbivore interactions*

Herbivores are dependent on plants for food, removing more than 20% of annual net primary productivity across biomes, habitats and natural and managed systems (Agrawal 2011).

Herbivory reduces a plant fitness through the removal of plant organs, such as leaves, responsible for photosynthesis (Marquis 1984). This in turn leads to the selection of plant defences (Rausher and Simms 1989). Most plants can tolerate some herbivory without a reduction in fitness, and some species or populations can tolerate a great deal of herbivory (McNaughton 1983, Paige and Whitham 1987).

1.2.1 *Plant defences*

As a first level of defence, a barrier against herbivores can be provided by a plant's physical structure. Physical or mechanical defences include leaf toughness, trichomes, thorns, and wax (Schoonhoven et al. 2005, Hanley et al. 2007). Plants may also invest in the production of a variety of defensive chemicals, also referred to as secondary metabolites (Schoonhoven et al. 2005). These include terpenoids, such as polygodial (Asakawa et al. 1988), nitrogen-based compounds, such as alkaloids (McKey 1974) and cyanogenic glycosides (Jones 1962), phenolics and tannins (Feeny 1970), and the polyacetates (Guillet et al. 1997).

Although each class of chemical compound is generally widespread, particular compounds within these classes may be specific to a particular grouping of plants. For example, terpenoids are the largest group of secondary metabolites but digitoxin, a type of cardenolide within the terpenoids, is only found in the genus *Digitalis* comprising about 20 species (Agrawal et al. 2012). Equally, mechanical defences are not uniquely isolated to any

particular plant group (Schoonhoven et al. 2005, Hanley et al. 2007). Differences in defensive traits in some instances may be associated with different types of herbivory. For example, vertebrate browsed plants may be more likely to possess traits that promote “tolerance” to herbivory, allowing for rapid regrowth, rather than “resistance”, such as chemical defences associated with invertebrate damage (Kotanen and Rosenthal 2000). However, as plants simultaneously employ a range of strategies and traits (Agrawal and Fishbein 2006), plant defensive strategies are likely to be coevolved interactions between a plant and its specific suite of herbivores (Tanentzap et al. 2011).

The production of defences for plants is thought to be costly, either directly due to the reallocation of resources to defence, or indirectly through reduced fitness trade-offs that influence interactions with other species (Strauss et al. 2002). Induced defences can help to reduce these costs by ensuring defensive compounds are only produced after a herbivore attacks (Agrawal 1998, Karban 2011). Alternatively, defences may always be present, known as constitutive defences. These are costly as they continually require resources, but the advantage is that they offer immediate defence against herbivores (Karbon et al. 1999). Studies have suggested that there is a trade-off between the two groups of defences (Koricheva et al. 2004). One hypothesis proposes that if plants have a high probability of being attacked, a plant should rely on constitutive defences, while if the probability of herbivory is low, defences should be induced (Karbon and Myers 1989). Alternatively, the ‘optimal defence’ hypothesis predicts that, where resources are limited, plants will invest protection in plant parts that are most valuable (McKey 1974), such as new leaves which are rich in nitrogen content and have yet to develop mechanical defences (McKey 1974, Stamp 2003).

There is debate about what drives the diversification of plant defences both for secondary chemicals (Hartley and Jones 1986) and structural/mechanical defences (Edwards 1989). The coevolution theory posits that herbivory pressure on plants has fuelled an ongoing co-evolution between plants and animals; plants have evolved a range of defensive strategies and traits to reduce the impact of herbivores, while herbivores evolve counter measures to overcome these increased defences (Ehrlich and Raven 1964, Karban and Agrawal 2002, Agrawal 2011). Alternatively, rather than as a response to herbivory, the primary function of many defensive traits may have been in response to other biotic and abiotic stressors (Jermy 1976, Edwards 1989). For example, divaricate plant architecture, a woody plant morphology of interlacing wiry stems with small internally located leaves, may have evolved to deter

herbivory from now extinct ratite herbivores (Bond et al. 2004, Bond and Silander 2007). On the other hand, it also reduces water loss (McGlone and Webb 1981) and confers tolerance to wind and frost within the crown interior (Darrow et al. 2001). It is argued however, that a trait can be viewed as defensive even if its primary function is not defence (Strauss and Agrawal 1999).

1.2.2 *Herbivore foraging*

Herbivores are often classed as specialist or generalist based on their foraging strategies. Specialists focus on one particular species or grouping of related plants, while generalists forage on a wide variety of species. The specialist-generalist herbivore hypothesis is used to explain the evolution of some plant defensive traits. Specialists are less impacted by high levels of a given plant defence compared to a generalist, but generalists should be able to tolerate an array of low level plant defences (Whittaker and Feeny 1971).

However, plants must often defend themselves against a diverse range of herbivore species including generalists and specialists. Although high levels of a chemical defence may deter a generalist herbivore, they can also be used by specialist insect herbivores as a host-finding signal for feeding and oviposition (Da Costa and Jones 1971, Macel and Vrieling 2003, Nieminen et al. 2003). For example, a specialist insect herbivore of *Brassica nigra* is attracted to those plants that contain high concentrations of its primary chemical defence, sinigrin, a glucosinolate secondary metabolite. However, generalist herbivores of *B. nigra* are deterred by this compound, and damage is negatively correlated with the concentration of sinigrin (Lankau 2007).

In contrast to herbivorous insects that mostly feed on only one plant family or genus, specialisation is relatively uncommon in vertebrate herbivores (Freeland and Janzen 1974, Bernays and Chapman 1994, Dearing et al. 2000). Generally, herbivores with a large body mass require more food, therefore herbivores of higher body mass ingest diets of lower quality (Clauss et al. 2013). Also, the larger the herbivore the bigger the bite size and therefore the amount of plant material ingested (Wilson and Kerley 2003). Research on the feeding choices of foraging mammals shows that while they prefer to select foods rich in nutrients and low in toxic chemical defences (Singer et al. 2002, Baraza et al. 2005, Dearing et al. 2005, Marsh et al. 2007), they often browse on multiple plant species that vary in nutritional and toxin content (Villalba and Provenza 2009). Mammalian herbivores will ingest these secondary metabolites when the cost of ingesting the toxin is outweighed by the

benefit of the nutrients acquired (Freeland and Janzen 1974, Provenza 1995, Duncan and Young 2002, Dearing et al. 2005, Marsh et al. 2005, Villalba and Provenza 2005). Therefore, mammalian herbivore foraging strategies can be many and complex (Shipley et al. 2009), and the specialist-generalist hypothesis is better thought of as a continuum (Poisot et al. 2015), especially for larger vertebrates.

1.2.3 Impact of invertebrate and vertebrate herbivory

As mentioned above (section 1.2.2), the level of impact one vertebrate herbivore can have on an individual plant can be large due to their increased body size compared with invertebrates. However, invertebrate herbivory involves the gradual removal of small amounts of tissue over a prolonged period of time. Therefore, plants likely face a continuous drain of resources from across several locations within the plant. The targeted, or narrow range of damage can mean that even a relatively minor level of tissue loss can have a disproportionately large impact on the plant (Kotanen and Rosenthal 2000). However, this targeted damage associated with invertebrate herbivory means that plants can direct their defences to particularly vulnerable areas and, given the slower rate of inflicted damage, allows the plant time to respond with increased (inducible) defence or regrowth (Endara and Coley 2011). Vertebrate herbivory, in contrast, is often sudden and severe, and a reflection of their large body size relative to that of the plants they consume (Kotanen and Rosenthal 2000). The occurrence of damage is often spatially and temporally stochastic (Varnamkhasti et al. 1995, Wang et al. 2006) and therefore in some instances, can exert a more significant impact on plant individuals and/or populations than attacks by invertebrate herbivores (Crawley 1989; however see Maron and Crone 2006).

1.2.4 Foraging Theory

Foraging for food costs a herbivore time and energy. Therefore, theory predicts that an animal will optimise a payoff from their foraging decisions, i.e. maximise energy gain for the lowest cost while foraging (Stephens and Krebs 1986). Optimal foraging theory helps us understand how an animal behaves when searching for food. A variation on this foraging model is the optimal diet model, which consists of three relatively robust predictions: (i) foragers will prefer prey that yield more energy per unit handling time; (ii) as the abundance of higher value prey increases, lower value prey should be dropped from the diet; and (iii) foragers should obey a quantitative threshold rule for when specific prey types should be included or excluded from an optimal diet, i.e. trade-off between maximizing the intake rate

while eating and minimising the search interval between prey (Pulliam 1974, Werner and Hall 1974, Charnov 1976). The optimal diet model works well at explaining the preferences and changes in preferences with varying abundance for foragers such as herbivores, but not so well for mobile prey where prey behaviour changes the dynamics of the model (Sih and Christensen 2001).

1.3 Learning about food

While herbivores can learn to use signals and cues to select desirable food when foraging (Freeland and Janzen 1974, Singer et al. 2002, Dearing et al. 2005, Marsh et al. 2006), the cognitive process of herbivores, especially mammalian herbivores, has received relatively little attention. How they decide which signals and cues of a plant to use, and how, when, and what factors affect how they learn about these signals and cues are unknown. However, studies in other foraging systems are often successfully applied across systems (Skelhorn et al. 2016). Below, I discuss the use of visual signals and cues by animals from the predator-prey foraging literature.

1.3.1 Predator-prey learning

How a predator learns about its prey is important in understanding the foraging dynamics between the animal and its food. In a process referred to as predator avoidance learning, predators can learn to avoid defended prey items over repeated encounters through association of the unpalatability of the prey, i.e. toxicity, with its warning signals (Berenbaum and Miliczky 1984, Sillén-Tullberg 1985, Lindström et al. 2001b, Ham et al. 2006, Skelhorn and Rowe 2006a, Prudic et al. 2007). The ability of a predator to learn is influenced by their cognitive and sensory abilities and therefore the selective pressures on prey signals are a product of those same sensory and cognitive abilities (Schaefer and Ruxton 2009, Skelhorn et al. 2016). Likewise, predators gain knowledge about defended prey when they attack them (Skelhorn and Rowe 2007, Halpin et al. 2014), and they also evaluate the costs and benefits of attacking palatable prey that vary in nutritional value (Simpson et al. 2004, Raubenheimer et al. 2007). This knowledge enables predators to make adaptive foraging decisions.

1.3.2 Use of visual signals or cues to avoid unpalatable prey

The definition of a signal is a trait or traits that convey information to other organisms and are maintained by natural selection. Cues, on the other hand, are defined as informative traits that evolved for reasons other than communication (Otte 1974). If an animal learns to avoid

traits associated with unpalatability and these traits were not initially associated with communication, they may be co-opted to become new communication signals, a process known as ritualization (Tinbergen 1952, Huxley 1966, Lorenz 1966). It is well recognised that prey that invest significantly in defences against predators generally advertise this defence through conspicuous or aposematic signals (Wallace 1867, Darwin 1871, Poulton 1890, Cott 1940). The information is of mutual interest to both the signaller (prey) and receiver (predator), as the signals expression is beneficial for both recipients (Maynard Smith and Harper 2003, Ruxton et al. 2004).

However, predators learn about defended prey over successive encounters. Signals that are more conspicuous are more readily associated with toxicity, reducing the learning time involved (Skelhorn and Rowe 2006b, Halpin et al. 2008, Aronsson and Gamberale-Stille 2013). Predators may generalise their learned avoidance of a warning signal to another similar signal (Mappes and Alatalo 1997, Gamberale-Stille and Tullberg 1999). For example, great tits (*Parus major*) were taught to discriminate between red, yellow, orange and grey palatable and unpalatable food items. However, birds that were initially trained that orange was palatable, after having been trained to avoid red and yellow, would now eat fewer orange prey items even though they were palatable (Ham et al. 2006). The generalisation of a signal can broaden as the negatively associated experience with a warning signal increases in strength (Duncan and Sheppard 1965, Lindström et al. 1997). Predators are also likely to generalise more within complex environments where the amount of stimuli requiring processing increases. Stimuli processing can be costly in terms of time and energy lost and therefore the predator faces associative trade-off decisions (Beatty et al. 2004, Lindstedt et al. 2011).

1.3.3 Use of visual warning signals by prey

Uniformity of warning signals, both within and across species, is assumed to increase their efficacy as the costs to potential prey of educating naïve predators are shared across individuals with similar signals (Müller 1879, Beatty et al. 2004, Rowland et al. 2007). Yet variation in the strength of warning signals exists between and within prey species when there are trade-offs in associative fitness benefits and costs. These trade-offs may come as a response to variation in environmental pressures (Hazel 2002, Sword 2002, Lindstedt et al. 2008a). For example, predators of the aposematic wood tiger moth (*Parasemia plantaginis*) larvae learn to avoid larvae with large signals faster than those with small signals (Lindstedt

et al. 2008b). However, larvae with small signals develop faster in low temperatures, suggestive of a thermoregulatory benefit (Lindstedt et al. 2009). Likewise, the variation in bumblebee colour patterns may be attributable to thermoregulation and predation risks (Williams 2007). Life history constraints can also affect the quality of signal expression. For example, the lack of availability of a quality diet reduces the ability to produce certain warning signal elements in *Parasemia plantaginis* moth larvae (Ojala et al. 2007). The complexity of the environment may also influence the requirement for signal uniformity. In simple environments less signal variation might be expected due to fewer interactions requiring trade-offs, but in complex environments with more interactions, the signal variation would be greater (Lindstedt et al. 2011).

1.3.4 Non-aposematic visual signals

Camouflaged prey are more common than conspicuous prey (Endler 1991), and not all defended prey are maximally aposematic or conspicuous (Endler and Mappes 2004, Tullberg et al. 2005). As long as prey is distinct from other prey, even cryptic prey can be recognised when detected and avoided (Sherratt and Beatty 2003, Merilaita and Ruxton 2007). Merilaita and Ruxton (2007) used theoretical modelling of predator prey detection and attack decision making in combination with an evolving virtual prey to understand the relative importance of conspicuousness against background, and the distinctiveness from other potential prey. Their results showed that “conspicuousness may result from selection for distinctiveness, but that selection for distinctiveness does not result in maximisation of conspicuousness”, and that “the relative importance of the two selective forces (for conspicuousness and for distinctiveness) will differ on a case-by-case basis”.

Conspicuous prey can draw unwanted attention making them more vulnerable to attacks by naïve (Lindström et al. 2001a, Riipi et al. 2001, Lindstedt et al. 2008b), or specialised predators (Yosef and Whitman 1992). Predators will seek out aposematic prey when the benefit of gaining nutrients out-weigh the costs of ingesting the defences (Villalba and Provenza 2005, Halpin et al. 2014). Therefore defended prey may benefit from being less conspicuous. However, there are morphological constraints and physiological costs imposed by camouflage (Ruxton et al. 2004).

1.3.5 Predator attacking strategy

Skelhorn et al. (2016) point out in their review on animal aposematic prey learning that to understand predators’ decisions on prey selection, we should think about how predators learn

about, rather than to *avoid*, aposematic prey in order to make adaptive foraging decisions. Decisions to attack one particular prey type are determined not only by the costs and benefits of attacking that prey type but also by the costs and benefits of attacking other available prey types (Merilaita and Kaitala 2002, Lindström et al. 2004, Skelhorn and Rowe 2005, Halpin et al. 2017).

1.4 Dishonest defensive signals

Deception as an anti-predator adaptation is a relatively common and well documented phenomenon in the animal kingdom, and occurs through the transmission of misinformation from prey to predator (Ruxton et al. 2004). Predator deception may work at a conscious or unconscious level within a signal receiver. Crypsis and camouflage work by avoiding detection from a searching predator by blending in with the background (Stevens and Merilaita 2009a, 2009b). The other form of deception is mimicry. In the broadest definition of mimicry, it can include deceptive strategies such as masquerade. This is where prey appear to mimic objects that are either innocuous or inedible by their predators, such as stones or twigs (Endler 1981, Skelhorn et al. 2009). However, the most commonly recognised form of deception comes in the form of Batesian mimicry. This is where an undefended species avoids predation by mimicking the warning signal of a defended species, proposed by Henry Bates in 1862 (Bates 1862).

1.4.1 Batesian mimicry

There are three protagonists in a mimicry complex: the ‘mimic’, a palatable species which mimics the defensive signal of a second species, the ‘model’, and the ‘signal-receiver’, who is duped by misidentifying the mimic as a model, conferring a fitness advantage on the mimic (Vane-Wright 1980). It is, therefore, the signal receiver that selects for the signal imitation in the mimic. Whilst selection on uniformity in warning signals amongst defended prey is hypothesised, variation exists within and between signals due to trade-offs in associative fitness benefits and costs (see section 1.3.3). Likewise, it is assumed that mimics always experience selection to more closely resemble their models (Ruxton et al. 2004), yet mimics are rarely exact replicas of their models, and imperfect mimicry is common (Kikuchi and Pfennig 2013).

1.4.2 Imperfect mimicry

Batesian mimics also face trade-offs, which can affect the degree to which they match their model's signal. Yet, successful mimicry requires that the mimic and model converge in the perceptual world of a predator such that it cannot easily distinguish between the two (Schaefer and Ruxton 2009), thus creating a level of uncertainty. How a signal receiver generalises over prey signals or cues (see section 1.3.2) is important for the evolution of Batesian mimicry (Lindström et al. 1997, Lynn et al. 2005, McGuire et al. 2006, Rowland et al. 2007, Kikuchi and Pfennig 2010a, 2010b, Iserbyt et al. 2011, Penney et al. 2012, Ihalainen et al. 2012). Below, I discuss several hypotheses highlighted by Kikuchi and Pfennig (2013) for the evolution of Batesian mimicry.

1.4.3 Imperfect mimicry hypotheses with empirical support for Batesian mimicry

1.4.3.1 Relaxed selection hypothesis

The risk to the signal receiver of accidentally attacking a model is high, so mimics are under very little selective pressure to improve their mimicry accuracy. The accuracy of the mimic to model is under a positive frequency-dependent selection with the abundance of other mimics. That is, as the ratio of mimics increase relative to models, the level of required signal matching to dupe the signal receiver increases. However, with the availability of alternative prey, or as the toxicity of the model increases, accuracy is under a negative frequency-dependency (Schmidt 1958, Duncan and Sheppard 1963, Sherratt 2002, Penney et al. 2012). This hypothesis has received widespread empirical support across different taxa (Pfennig et al. 2001, Darst and Cummings 2006, Iserbyt et al. 2011). Negative selective pressure with increasing alternative prey has also received support, but only from trained birds and artificial prey (Lindström et al. 2004). Below I discuss some of the key predictions of this hypothesis:

Fundamental elements of this hypothesis were recognised by Bates (1862), who argued that only when mimics were significantly outnumbered by models could imitation be of any advantage. The frequency-dependency hypothesis suggests that selection for the accuracy of mimicry is relaxed as the proportion of models increases, and likewise, if mimics are too common the protection through predator deception breaks down as predators learn to ignore the warning signal (Pfennig et al. 2001, Ruxton et al. 2004). The unprofitability of the model can also relax selection on the level of accuracy. As the cost associated with the model increases, selection on perfect mimicry decreases due to stronger avoidance measures from the signal receiver (Lindström et al. 1997, Sherratt 2002). However, if a model is too

unprofitable, this may cause a breakdown in the signal receiver's avoidance-learning, and therefore an intermediate level of protection is most effective for mimic protection (Holen 2013). When the availability of alternative prey is high, it is simply easier for a signal receiver to attack the alternative prey rather than discriminate between mimics and models. This also reduces selection on perfect mimicry (Hetz and Slobodchikoff 1988, Lindström et al. 2004, Penney et al. 2012).

1.4.3.2 Eye-of-the-beholder hypothesis

Humans mistakenly attribute characteristics that are not relevant for the intended signal receiver (Cuthill and Bennett 1993, Dittrich et al. 1993). In this instance, imperfections are either not relevant, or in some cases, the imperfect mimicry is not mimicry at all. Evidence for this hypothesis can be found in the misalignment of the order of coloured bands around venomous coral snakes and the mimicking nonvenomous scarlet snakes in the south-eastern United States. Under selection are the proportions of colours (Harper and Pfennig 2007), and the general ringed appearance (Pfennig et al. 2001), but not the order of the coloured rings (Kikuchi and Pfennig 2010b), therefore the imperfect mimicry is human perception. Taking into account the cognitive and sensory capabilities of the target signal receiver through modelling, can help explain the human perceived inaccuracies (Penney et al. 2012).

1.4.3.3 Chase-away hypothesis

Models are selected to evolve away from their mimics due to signal receivers mistaking them for mimics. Mimics are less accurate due to the time-lag with their response (Nur 1970, McGuire et al. 2006, Franks et al. 2009). It is possible that conspicuousness in some salamanders may have evolved in order to appear distinct from their Batesian mimics and avoid misidentification by avian predators (Kraemer et al. 2015). Theoretical models predict that signals associated with defence will always evolve towards distinctiveness due to selection pressures (Merilaita and Ruxton 2007). However, if the costs of misidentification on a model are relatively low, or if the motivation of the signal receiver to seek out mimics is low, then selection pressures on the model to be distinct from the mimic is low.

1.4.3.4 Mimetic breakdown hypothesis

A lack of models reduces the protection from the signal receiver, and therefore causes the mimicry system to breakdown (Sheppard 1959, Brower 1960, Pfennig and Mullen 2010). This has also been demonstrated between scarlet kingsnakes and coral snakes that occur in

sympatry and allopatry. When the two species are found together, increased mimicry is selected for, while in the absence of models, mimics are attacked more (Pfennig et al. 2001, 2007), and the colour pattern becomes less similar. The persistence of the mimic phenotype in allopatry is due to migration (Harper and Pfennig 2008).

In the absence of models, mimics might experience higher than expected predation (Pfennig et al. 2007). A conspicuous signal may draw too much attention, so mimics evolve towards a more cryptic phenotype (Joron 2008). Likewise, if a mimetic trait has physiological disadvantages, in the absence of the model that trait should be selected out of the population due to its reduced fitness effect. Whilst I cannot find any examples of this scenario within Batesian mimicry, an example of an aposematic signal that has a physiological disadvantage can be found in the aposematic signalling wood tiger moth (*Parasemia plantaginis*) larvae. The strength of the warning signal can constrain their thermoregulatory abilities (Lindstedt et al. 2009). This example could be extrapolated out to a hypothetical Batesian mimicry system where, in the absence of the model, the physiological disadvantage of the same warning signal alongside the increased predation pressure on the mimic would drive the warning signal phenotype out of the population.

1.4.3.5 Multiple predators hypothesis

Mimics trade-off between duping generalist predators and avoiding specialist predators which causes opposing selective pressures on the mimic's phenotype (Pekár et al. 2011). There is evidence for this hypothesis within ant-mimicking spiders. The least accurate mimics still gain protection from predators that avoid ants, but are faster at escaping from ant predators than are the ant-mimics that are of higher accuracy (Pekár et al. 2011).

To some degree it is likely all mimics face this problem as almost all prey live with multiple types of predators (Sih et al. 1998). However, as discussed in the above sections, the types of predators (generalists or specialists), their sensory and cognitive capabilities, and how they forage will likely impact how a warning signal is expressed. It would be important to account for the fitness impact on prey of each predator when assessing this hypothesis.

1.4.3.6 Developmental and genetic constraints hypothesis

A mimic is unable to produce a more accurate mimetic signal due to being constrained by an intrinsic inability (Maynard Smith et al. 1985). Very few studies have examined this hypothesis. However, there is some evidence that in order to produce the shared signal, some

models and mimics use at least some of the same genes and/or physiological pathway (Ford 1953, Kikuchi and Pfennig 2012).

1.4.4 Imperfect mimicry hypotheses with no empirical support for Batesian mimicry

A number of hypotheses highlighted by Kikuchi and Pfennig (2013), to date, have no empirical support. Below I discuss these in relation to Batesian mimicry.

1.4.4.1 Perceptual exploitation hypothesis

In the perceptual exploitation hypothesis, the signal receiver has a cognitive or sensory bias towards a characteristic that is outside of the model's signal. Therefore, the imperfect mimics elicit a stronger avoidance response than their models (Vereecken and Schiestl 2008).

Compared with mimicry, perceptual exploitation is argued to be a simpler mechanism (Schaefer and Ruxton 2009). Perceptual exploitation takes advantage of the innate response to a particular stimuli. Therefore, Schaefer and Ruxton (2009) argue that the one key distinction between mimicry and perceptual exploitation is that “only mimicry predicts that animals confuse one species (the mimic) with another one (the model)”. In many animal species, sensory biases include the generalised preferences for larger sizes in mates (Ryan and Keddy-Hector 1992), and cognitive biases arise from generalisation and peak shift phenomena that arise during associative learning of signals with rewards (Chittka and Raine 2006, ten Cate and Rowe 2007). As it is important how a predator generalises signals for the evolution of Batesian mimicry (Lindström et al. 1997, Lynn et al. 2005, McGuire et al. 2006, Rowland et al. 2007, Kikuchi and Pfennig 2010a, 2010b, Iserbyt et al. 2011, Penney et al. 2012, Ihalainen et al. 2012), exploitation of perceptual biases may be an important precursory step for the evolution of mimicry in some systems (Schaefer and Ruxton 2009).

1.4.4.2 Satyric mimicry hypothesis

The satyric mimicry hypothesis predicts that a blend of different components of the model's signal with other irrelevant characteristics cause confusion in the signal receiver. This increases the level of protection over other imperfect mimics (Howse and Allen 1994).

Disruptive patterns, which break up the recognisable shape of the prey (Fraser et al. 2007, Kelman et al. 2007), along with a similar idea of “dazzling” or distractive markings, where detection of prey is made difficult by manipulating the attention of the predator, may aid prey as a defensive tactic (Dimitrova et al. 2009). As the cognitive process of confusion has been

demonstrated, it may be possible that mimicry complexes exist where this hypothesis is applicable.

1.4.4.3 Multiple models hypothesis

In the multiple models hypothesis, mimics take on an intermediate phenotype between models when multiple models exist. This “covering all bases” technique offers greater protection than mimicking the signal of just one model (Edmunds 2000, Sherratt 2002). This hypothesis relies on the physiological and production fitness costs of the mimetic signal being outweighed by the overall fitness cost of predation. However, fitness costs from predation can vary, especially in plants (Paige and Whitham 1987). If the costs of producing this intermediate level of signal are too great, it would be more advantageous to pick just one model.

1.4.4.4 Kin selection hypothesis

The kin selection hypothesis predicts that the fitness on the population of related mimics increases when mimicry is imperfect, especially as models become rare relative to mimics (Johnstone 2002, Penney et al. 2012). The weakest mimic takes a hit for the “team”, leaving their more accurately mimicking kin to keep reproducing. As the name suggests, this hypothesis should be more prevalent in species with limited dispersal and high degrees of family grouping (Johnstone 2002). As Kikuchi and Pfennig (2013) highlighted, to date no studies have explicitly measured relatedness within any natural population of mimics.

1.4.4.5 Character displacement hypothesis

Under the character displacement hypothesis, the mimetic trait causes competition to increase between mimics and models. This in turn displaces the phenotypic optimum away from accuracy in the mimetic trait (Pfennig and Kikuchi 2012). This hypothesis is similar to the chase away hypothesis, but now the non-predation fitness effects likely outweigh the predation fitness effects. For Batesian mimics that occupy a similar ecological niche to their models, this is likely to impact models to some degree, especially as the resources they both compete for become rarer.

1.4.4.6 Speed-accuracy trade-off hypothesis

The speed-accuracy trade-off hypothesis postulates that difficult perceptual tasks require a lot of time. Time is a cost to a foraging animal, therefore they tend to generalise signals (Chittka

and Osorio 2007). As mentioned in the perceptual exploitation hypothesis, generalisation is an important function within Batesian mimicry. Optimal diet theory predicts that a foraging animal is unlikely to spend a significant amount of time distinguishing between similar prey, especially if there are alternative food sources available (Pulliam 1974, Werner and Hall 1974, Charnov 1976). Therefore, signal receivers are likely to incorporate speed-accuracy trade-off decisions in most mimicry complexes. While not included in the hypotheses highlighted by Kikuchi and Pfennig (2013), I have included it here because of how it relates to a number of foraging theory and Batesian mimicry concepts.

1.4.5 Multiple hypotheses in a single system

Kikuchi and Pfennig (2013) highlight the need to determine the importance of the relative contribution of each hypothesis to the evolution of imperfect mimicry, and that the best way to do so is in a complex in which multiple hypotheses can be evaluated simultaneously. To date, studies are lacking. Results from the complexes that have been subjected to multiple-hypothesis testing indicate that different hypotheses are not mutually exclusive within a single complex. For example, the scarlet kingsnake and coral snake mimicry complex in the south-eastern United States used as examples in three of the above hypotheses: the relaxed selection hypothesis, the mimetic breakdown hypothesis, and the eye-of-the-beholder hypothesis, which can all explain the apparent imperfect mimicry.

1.5 Leaf shape and colour influences consumption and performance

Brown and Lawton (1991) proposed that if leaf shape and size influence the impact of herbivores, it would be open to selection and therefore herbivores may be, in part, responsible for some of the variety in leaf shapes and sizes. Leaf shape has been demonstrated to influence the ovipositioning choices of insect herbivores. For example, the pipevine swallowtail butterfly (*Battus philenor*), small grass yellow (*Eurema brigitta*) and pink grass yellow (*E. herlauses*) butterflies use leaf shape to search for suitable larval host plants for ovipositioning, alighting on plants with similar leaf shapes to their preferred host plant (Rausher 1978, Mackay and Jones 1989). Leaf shape can also affect the consumption and performance of insect herbivores. For example, the alternative leaf morphs of ivy-leaved morning glory (*Ipomoea hederacea*) affects the performance and consumption of several insect species, but how they do this differs between species (Campitelli et al. 2008a). It can also provide a visual cue of a plant's palatability. For example, *Heliconius erato* butterflies

were able to be conditioned to oviposit on either their natural larval host (*Passiflora biflora*), or a different shaped *Passiflora* spp. leaf (Dell'aglio et al. 2016).

Leaf colouration may also help plants deter herbivores. Variegated leaves, that is, leaves with areas of reduced or obstructed chlorophyll producing a whitish pattern, suffer less herbivory than non-variegated leaves in natural populations of *Hydrophyllum virginianum* (Campitelli et al. 2008b). Variegations may reduce herbivory on plants because variegated leaves may appear to insect herbivores as already damaged by other insects (Soltau et al. 2008).

Anthocyanins can function as camouflage in leaves, making plants cryptic to herbivores by blending the plant in with the background of leaf litter and plant detritus (Fadzly et al. 2009, Klooster et al. 2009, Fadzly and Burns 2010). They can also act as an honest signal in the leaf margins to insect herbivores of the level of defensive investment in a leaf (Cooney et al. 2012, Hughes and Lev-Yadun 2015). Autumnal red anthocyanic flushes of whole leaves are hypothesised to signal increased defences and/or low nutrient quality to egg-laying insects (Hamilton and Brown 2001, Archetti and Brown 2004, Archetti 2009b).

1.6 Batesian mimicry in plants

The evolution of defensive mimicry in plants is theoretically possible, and may even be relatively common, depending on how reluctant a herbivore is to eat again after sampling a defended plant (Augner and Bernays 1998). Augner and Bernays (1998) found that mimicry could evolve if a herbivore shows any avoidance of individuals displaying a signal of unpalatability. In contrast, if a herbivore always samples a signalling defended plant, whether it is an honest signal or not, mimicry would never evolve. Whilst some thorn-less plants may mimic the presence of aposematic thorns (Lev-Yadun 2003), amongst the background of leaf morphological heterogeneity exist examples in which the leaf shape, size and colour of one species correlates closely with that in another, unrelated species. This convergence in leaf shape has been hypothesised to present a potential fitness advantage by using whole leaf mimicry to evade detection by herbivores.

1.6.1 Postulated cases of defensive plant-plant mimicry – a review

Within the flora of New Zealand, the use of thorns and spines as a mechanical defensive strategy are relatively uncommon. However, within the tussock-like members of the genus *Aciphylla*, just over half of the 30 species are thought to have evolved sharp points on the end of their leaves to deter browsing (Wallace 1889, Atkinson and Greenwood 1989). The non-spiny-leafed *Celmisia petriei* and *C. lyallii* look like spiny members *Aciphylla* genus that

they grow alongside of (Atkinson and Greenwood 1989, Brown and Lawton 1991). The non-stinging nettle like group of plants found throughout Europe containing species from across several genera (*Lamium album*, *L. purpureum*, *Lamiastrum galeobdolon*, *Ballota nigra*, *Galeopsis* spp.), which are commonly known as dead nettles because of their lack of sting, resemble the well defended European stinging nettle (*Urtica dioica*) (Brown and Lawton 1991, Wheeler 2004).

In other cases, this correlation in resemblance only seems to occur in the presence of another particular plant species. In the heavily grazed desert–steppe transition zone of the northern Negev, Israel, the leaf form and overall habit of the hill ecotype of *Iris atrofusca* appears to be more similar to that of the unpalatable *Asphodelus ramosus*, than to the valley ecotype growing in the absence of *A. ramosus* (Shimshi 1979). Leaves on the woody vine *Boquila trifoliolata* of southern Chile, bear striking physical resemblance to those on several host trees; when portions of the vine traverse other hosts, their leaf shape changes accordingly (Gianoli and Carrasco-Urra 2014). Similarly, the leaves of some Australian mistletoes closely resemble those of their host plants in leaf colour and general shape (Barlow and Wiens 1977, Ehleringer et al. 1986, Canyon and Hill 1997).

In the above instances of visually similar leaf morphologies, it is not known whether a herbivore common to both species avoids the purported mimic due to this perceived similarity with its model. Therefore, it is not known whether the similarity confers a fitness advantage on the mimic, a requirement for Batesian mimicry (Vane-Wright 1980). In all but the vine study by Gianoli and Carrasco-Urra (2014) and the Australian mistletoes, the similarity in leaf morphology is anecdotal and has not been quantified. While in the case of the Australian mistletoes, only a simple quantification of leaf shape using length by width measurements has been undertaken and some of these similarities between host and mistletoe are also shared with other species in the surrounding plant community (Blick et al. 2012). It is therefore likely that herbivores would use different features to discriminate between host and mistletoe (Canyon and Hill 1997).

1.6.2 Human-induced mimicry in plants

Humans are thought to be responsible for a form of mimicry associated with crop plants and their weeds known as Vavilovian mimicry. In these instances, the receivers are humans or agricultural machinery used to remove unwanted plants or seeds from a farmed crop (Wickler 1968). Although Vavilovian mimicry is a type of Batesian mimicry, rather than being

defended, the model species is a desirable species and the fitness advantage to the mimic comes from duping humans into thinking it too is desirable. For example, barnyard grass (*Echinochloa oryzoides*) that looks similar to rice plants (*Oryza sativa*), escape unnoticed during manual weeding of rice paddies at the seedling stage. The seeds are later mixed with rice seeds and those of *E. oryzoides* individuals that went unnoticed due to being visually similar to *O. sativa* during the weeding stage are replanted (Barrett 1983). Therefore, through weeding, *E. oryzoides* has increased its resemblance to *O. sativa*. However, the increase in the use of herbicides has resulted in *E. oryzoides* being replaced with a more herbicide-resistant barnyard grass (*Echinochloa crus-galli* var. *crus-galli*) that does not share the same visual resemblance to rice (Yamasue 2001).

1.7 Gap in knowledge

Although Batesian mimicry is relatively common in the animal kingdom (Ruxton et al. 2004), successful plant mimicry requires that the mimic and model converge in the perceptual world of an approaching herbivore such that it cannot easily distinguish between the two (Schaefer and Ruxton 2009). Unequivocal proof of mimicry requires evidence that: (i) both the mimic and a known model are attacked by the same herbivore; (ii) the herbivore is unable to distinguish between them; and (iii) this confusion increases the fitness of the mimic. To date, no study has satisfied these three criteria in plants (Schaefer and Ruxton 2009, 2011).

1.8 *Alseuosmia pusilla* and *Pseudowintera colorata* are model species to test for Batesian mimicry

Our focal plants, *Alseuosmia pusilla* (Colenso) A. Cunningham (Alseuosmiaceae) and *Pseudowintera colorata* (Raoul) Dandy (Winteraceae) are understory shrubs found in conifer-broadleaf and beech forests of New Zealand. Horopito (*P. colorata*) is found throughout the North and South Islands, while small toropapa (*A. pusilla*) is not nearly as widespread, distributed from the lower two-thirds of the North Island to as far south as the middle of the South Island, but only on the western side (approximately from S37 59.33, E175 5.53 to S43 13.25, E170 9.47). Together they possess several properties which makes them a particularly well suited study system to test for Batesian mimicry.

1. *A. pusilla* has attracted attention because leaf size, shape, and anthocyanin pigmentation make the leaves of individual plants at some locations indistinguishable, at least to the human eye, from those of an unrelated species, *P. colorata* with which

it is often sympatric (Fig. 2.1A) (Greenwood and Atkinson 1977, Dawson 1988, Atkinson and Greenwood 1989).

2. The leaves of *A. pusilla* contain few anti-herbivory compounds (Cambie and Parnell 1970) and are readily eaten by introduced ungulates (Greenwood and Atkinson 1977, Nugent et al. 2001); in contrast, those of *P. colorata* are rich in polygodial, a sesquiterpene dialdehyde that imparts a pungent taste and is a potent insect anti-feedent (Asakawa et al. 1988).
3. *P. colorata* leaves are also unpalatable to introduced herbivorous mammals (Forsyth et al. 2005), and, it has been suggested, may similarly have been avoided by moa (Greenwood and Atkinson 1977), the extinct flightless birds which are thought to have presented significant browsing pressure on New Zealand's flora over the last 65 million years (Atkinson and Greenwood 1989, Wood et al. 2013). It has been postulated that at locations where the two plant species co-occur, *A. pusilla* may escape predation by effectively functioning as a Batesian mimic of *P. colorata* (Dawson 1988, Atkinson and Greenwood 1989).

1.9 Aims of the thesis

The overall aim of this thesis was to comprehensively test the hypothesis that *A. pusilla* acts as a Batesian mimic of *P. colorata*. In order to do so, I aimed to satisfy the criteria for defensive Batesian mimicry in plants as proposed by Schaefer and Ruxton (2009, 2011) (see section 1.7).

1.9.1 Specific objectives

*1.9.1.1 Establish a spatially explicit method to quantify leaf shape similarity between *A. pusilla* and *P. colorata* [Chapter 2]*

Using a spatially explicit morphometric analysis, I tested how similar the leaf shape of *A. pusilla* and *P. colorata* were to each other and surrounding sympatric plant species. I hypothesised that: (i) leaf shape of *P. colorata* is sufficiently distinct from those of neighbouring plant species such that its morphology might serve to signal its unpalatability; (ii) the leaf shape is shared exclusively by the undefended *A. pusilla*; (iii) variation in *P. colorata* leaf shape across an environmental gradient is matched by variation in foliar morphology of *A. pusilla*; and (iv) leaf shape similarity is greater the closer the two species are to one another.

1.9.1.2 Test the influence of increased herbivory pressure on leaf shape variation between A. pusilla and P. colorata [Chapter 3]

Using the spatially explicit morphometric analysis established in Chapter 2 and field surveys of plant abundance, I tested how leaf shape variation was affected between *A. pusilla* and *P. colorata* and surrounding sympatric plant species when exposed to increased mammalian herbivory pressure. I hypothesised that at a field site with high mammalian herbivore density: (i) variation in leaf shape will be reduced in the unpalatable *P. colorata* but not in the surrounding sympatric species such that its morphology is acting as a stronger signal of its unpalatability; (ii) variation in leaf shape of the undefended *A. pusilla* will also decrease; (iii) variation in *P. colorata* leaf shape across an environmental gradient will not only be closely matched by variation in foliar morphology of *A. pusilla*, but also be of a comparable magnitude; (iv) *A. pusilla* will be more similar in leaf shape to the population of *P. colorata* rather than greater, the closer in distance the two species are to one another; (v) *A. pusilla* will increase in abundance in proportion to an increase in *P. colorata*; and (vi) the degree of similarity between *A. pusilla* and *P. colorata* will be the same as that of a field site with low mammalian herbivore density.

1.9.1.3 Test whether red deer will eat A. pusilla in the presence of P. colorata [Chapter 4]

I conducted a field feeding trial using captive red deer, a relatively common introduced mammalian ungulate in New Zealand forests that is known to avoid *P. colorata*, to test the hypotheses: (i) *P. colorata* is avoided by deer more than *A. pusilla*, (ii) as the proportion of *P. colorata* increases in relation to *A. pusilla*, *A. pusilla* individuals will suffer less herbivory, and (iii) known palatable species that do not share a similar leaf shape and colour with *P. colorata* are readily eaten in the presence of *P. colorata*.

1.9.1.4 Test how leaf shape, leaf size and leaf colour are used as cues or signals to discriminate between A. pusilla and P. colorata [Chapter 5]

I conducted a computer-based survey using humans as surrogate herbivores to estimate how different leaf traits are used as cues or signals to discriminate between *A. pusilla* and *P. colorata* under different conditions. I investigated how subjects' discrimination abilities are affected by: (i) different leaf traits and how they interact; (ii) the variation in the overall similarity of leaf shape between *P. colorata* and *A. pusilla*; (iii) the frequency of models to mimics; (iv) the speed of feedback on selections; and (v) the level of conspicuousness of the leaves.

Chapter 2: The significance of shared leaf shape in *Alseuosmia pusilla* and *Pseudowintera colorata*

2.1 ABSTRACT

Leaf shape, size and colour are used by herbivores to identify sources of palatable foliage for food. It is possible, therefore, that an undefended plant might gain protection from herbivores by matching leaf characteristics of a chemically-defended species. I demonstrate the use of a geometric morphometric approach to quantify spatial variation in leaf shape and size across populations of *Pseudowintera colorata*, and its putative Batesian mimic, *Alseuosmia pusilla*. Using the Cartesian coordinates of leaf margins as descriptors of leaf shape, I found that in the chemically-defended *P. colorata*, leaves were morphologically distinct from all neighbouring species except for the undefended *A. pusilla*. *A. pusilla* individuals were more similar to neighbouring than to distant *P. colorata*, and 90% of leaf shape variation in the two species varied similarly across an elevational gradient. The data are consistent with Batesian mimicry, wherein the conspicuous characteristic of a defended model is replicated by an undefended mimic across its entire growing range. This study provides the first detailed and powerful quantitative evidence of leaf shape being matched between an undefended plant species to a chemically-defended unrelated species across a shared growing range, and highlights the importance of using a spatially explicit morphometric method when investigating leaf shape, especially in relation to plant mimicry.

2.2 INTRODUCTION

Many plant species exhibit morphological plasticity in response to ontogenetic, abiotic, competitive, and predatory pressures. These traits often fluctuate predictably across environmental gradients, suggesting that phenotypic plasticity confers a selective advantage to a plant in its natural habitat (Hovenden and Vander Schoor 2006, Royer et al. 2009). Leaf morphology, for example, assists in temperature regulation (Gurevitch and Schuepp 1990, Hegazy and El Amry 1998, Roth-Nebelsick 2001) and light interception (Horn 1971, Tsukaya 2005); it can also serve to minimise wind damage (Anten et al. 2010), to tolerate salinity (Sinclair and Hoffmann 2003), drought, or nutrient deficiencies (Cunningham et al. 1999, Leigh et al. 2011), or to deter predation by herbivores (Niemelä and Tuomi 1987, Brown and Lawton 1991). Within these broader trends, however, there is often considerable intraspecific variation in leaf shape even in the same environment (McDonald et al. 2003,

Nicotra et al. 2011). The possible functional significance of such variation is usually far from clear.

Amidst this background of morphological heterogeneity, there are examples in which variation in the leaf shape, size and colour of one species correlates closely with that in another, unrelated species. For example, the non-spiny leafed *Celmisia petriei* and *C. lyallii* from New Zealand look like spiny members of the genus *Aciphylla* (Brown and Lawton 1991), whilst the non-stinging nettle like group of plants containing species from across several genera (*Lamium album*, *L. purpureum*, *Lamiastrum galeobdolon*, *Ballota nigra*, *Galeopsis* spp.), commonly known as dead nettles due to their lack of sting, resemble the defended European stinging nettle (*Urtica dioica*) (Brown and Lawton 1991, Wheeler 2004). In other cases, this correlation of resemblance only seems to occur in the presence of a particular species. In the heavily grazed desert/steppe transition zone of the northern Negev, Israel, the leaf form and overall habit of the hill ecotype of *Iris atrofusca* appears to be more similar to that of the unpalatable *Asphodelus ramosus*, than to the valley ecotype growing in the absence of *A. ramosus* (Shimshi 1979). Leaves on the woody vine *Boquila trifoliolata* of southern Chile, bear striking physical resemblance to those on their host tree; when portions of the vine traverse other hosts, their leaf shape changes accordingly (Gianoli and Carrasco-Urra 2014). Similarly, the leaves of some Australian mistletoes closely resemble those of their host plants (Barlow and Wiens 1977, Canyon and Hill 1997). In those instances the convergence in leaf shape has been hypothesised to present a potential fitness advantage by using mimicry to evade detection by herbivores. Leaf shape is known to influence the consumption, performance, and ovipositing choices of insect herbivores, and thus may provide a visual cue of a plant's palatability (Rausher 1978, Mackay and Jones 1989, Rivero-Lynch et al. 1996, Campitelli et al. 2008a).

Successful plant mimicry requires that the mimic and model converge in the perceptual world of an approaching herbivore such that it cannot easily distinguish between the two (Schaefer and Ruxton 2009). It needs to be demonstrated, therefore, that leaf shape, size and colour of the putative mimic do indeed overlap with the ranges of those of the model, and that any morphological convergence is more evident between individuals where the two species co-occur. Previous studies of mimicry in vegetative shoots have relied on 'traditional' measures such as leaf length, width, area, and numbers of lobes, which, although useful as broad descriptors of morphology, cannot resolve subtle differences in shape that might be used as a visual cue by an approaching herbivore. Here, I use a spatially-explicit geometric

morphometric approach, i.e. taking into account how leaf shape varies both within and between species over distance, to quantify leaf morphology in two species from a natural forest population in New Zealand. Geometric morphometrics provide a higher resolution of captured shape information than do the more traditional measurements (Rohlf and Marcus 1993, Klingenberg 2010).

Our focal plant, *Alseuosmia pusilla* (Alseuosmiaceae), is an understory shrub found in conifer-broadleaf and beech forests of New Zealand. The species has attracted attention because leaf size, shape, and anthocyanin pigmentation make the leaves of individual plants at some locations indistinguishable, at least to the human eye, from those of an unrelated species, *Pseudowintera colorata* (Winteraceae), with which it is often sympatric (Fig. 2.1A) (Greenwood and Atkinson 1977, Dawson 1988, Atkinson and Greenwood 1989). The leaves of *A. pusilla* contain only a few anti-herbivory compounds (Cambie and Parnell 1970) and are readily eaten by introduced ungulates (Greenwood and Atkinson 1977, Nugent et al. 2001); in contrast, those of *P. colorata* are rich in polygodial, a sesquiterpene dialdehyde which imparts a pungent taste and is a potent insect anti-feedent (Asakawa et al. 1988). *P. colorata* leaves are also unpalatable to introduced herbivorous mammals (Forsyth et al. 2005), and, it has been suggested, may similarly have been avoided by moa (Greenwood and Atkinson 1977), the extinct flightless birds which are thought to have presented significant browsing pressure on New Zealand's flora over the last 65 million years (Atkinson and Greenwood 1989, Wood et al. 2013). It has been postulated that at locations where the two plant species co-occur, *A. pusilla* may escape predation by effectively functioning as a Batesian mimic of *P. colorata* (Dawson 1988, Atkinson and Greenwood 1989). However, this hypothesis lacks any empirical evidence.

Fig. 2.1. Leaf morphology of *Pseudowintera colorata* and *Alseuosmia pusilla*. (A) *P. colorata* (P.c) and *A. pusilla* (A.p) seedlings growing together under forest canopy (top), and in the open due to tree fall (bottom). (B) Selected examples to show the range of *A. pusilla*, and (C) *P. colorata* leaves across the elevational gradient. Scale bar = 20 mm.

I hypothesise that: (i) leaf shape of *P. colorata* is sufficiently distinct from those of neighbouring plant species such that its morphology might serve to signal its unpalatability; (ii) the leaf shape is shared exclusively by the undefended *A. pusilla*; (iii) variation in *P. colorata* leaf shape across an environmental gradient is matched by variation in foliar morphology of *A. pusilla*; and (iv) leaf shape similarity is greater the closer the two species are to one another.

2.3 MATERIALS AND METHODS

2.3.1 Plant Material

P. colorata and *A. pusilla* leaves were collected from a 4.5km stretch of track (S40 53.30, E175 14.22 to S40 54.46, E175 15.37) through montane forest in the Otaki Forks region of the Tararua Forest Park, New Zealand (see Appendix A.1). The collection area covered the full elevational range for the two species at this location (470 – 870 m), characterised by a shift from the lower conifer-broadleaf forest to the higher *Lophozonia*- and *Fuscospora*-dominated forest. *A. pusilla* grew along the entire transect; *P. colorata* was most common

above 720 m along the final 1.5 km of the transect, where it became the prominent understorey tree. Every *P. colorata* and *A. pusilla* within 2 m of one side of the track was sampled if the plants were between 15 cm and 2 m tall, and their location recorded using a Garmin 60 GPS (Olathe, USA; accuracy: ± 2 -10 m). The youngest fully expanded leaf was excised from a total of 211 *P. colorata* and 467 *A. pusilla* plants. When there were multiple branches, the youngest fully expanded leaf was removed from the highest branch.

In addition, we collected leaves from seven other tree and shrub species for leaf size and shape were similar to our focal species, and which were located within 1.5 m of *P. colorata* and *A. pusilla* plants. Between 60 and 130 individuals from each species were sampled; one leaf was collected from each individual from the closest branch facing the nearest *P. colorata* or *A. pusilla* plant. All leaves were refrigerated within 6 hours of collection.

Plant material collected was approved under Part IIIB of the Conservation Act 1987 and Section 49 of the Reserves Act 1977 with the New Zealand Department of Conservation permit number WE/25115/FLO.

2.3.2 Leaf Shape Capturing

Digital images of the adaxial surface of the leaves were captured using a Canon CanoScan 8400F flatbed scanner (Tokyo, Japan) at 600 dpi. Adobe Photoshop 5.0 (San Jose, CA, USA) was used to re-create the original leaf shape by filling in any areas lost to herbivory. If the herbivory damage was too extensive for a confident reconstruction, the original leaf shape was estimated from comparison with that of remaining adjacent lamina. Leaf shape was quantified by LeafAnalyser 2.3.0 (Weight et al. 2008) using the Cartesian coordinates of 70 evenly distributed landmarks distributed around the margin of each leaf. The coordinates were exported into R 2.13.1 (The R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis.

2.3.3 Leaf Colour

The reflectance spectra of green and red portions of a randomly chosen sub sample of *P. colorata* (red N = 5, green N = 8), and *A. pusilla* (red N = 4, green N = 9) leaves were recorded using an Ocean Optics (Dunedin, FL, USA) USB2000 spectrometer and with an HL-2000-CAL light source. Diffuse reflectance was measured relative to that of a white Teflon standard. The fibre optics probe was fixed at 45° to the leaf's adaxial surface and

spectra were measured at 0.2 nm intervals from 450–700 nm using the Ocean Optics SpectraSuite software.

2.3.4 Statistical Analysis

Cartesian coordinates were transformed into partial Procrustes coordinates using the software package PAST 2.17c (Hammer et al. 2001) to minimise differences in leaf orientation of the scanned images without scaling for leaf area. To quantify the similarity in overall leaf shape between *P. colorata*, *A. pusilla* and the surrounding plant species at the field site, the partial Procrustes coordinate residuals (i.e., tangent space coordinates) sourced from PAST 2.17c were used in a discriminant function analysis (DA) with IBM SPSS Statistics version 20 for Windows (Armonk, NY, USA). Using the full data set of all species as a training set, DA reclassified each individual leaf blindly into a species group based on how variation of leaf shape compared to the leaf shape of all individuals in the set.

Because the sampling site spanned an elevational gradient of 400m, we examined how variation in leaf shape between the two species altered with elevation. A principal component analysis (PCA) was run using the 70 partial Procrustes coordinates, reducing the number for each species into four principle components (PC) that explained the majority of shape variation for each leaf. Linear regressions for each PC as a function of elevation were compared for each species by ANCOVA.

To quantify spatial variation in leaf shape, we compared the Euclidean distances among *A. pusilla* and *P. colorata* plants on each PC plot with the geographical distances among them. To do this, the PC data were exported into R 2.13.1, whereupon the PC score of each *A. pusilla* or *P. colorata* was compared in turn to the PC scores of all individuals of the opposing species located within a 10m radius. The radial distance from the focal plant was progressively increased in 10m bands, and the process repeated until every individual of the opposing species across the sampling site had been compared. Using the log-likelihood function we tested the likelihood of how similar each individual's leaf shape was to those of the cohort of surrounding members of the second species at each 10m interval as compared with those of the remaining members.

2.4 RESULTS

2.4.1 Leaf Morphology

The leaf laminae of *A. pusilla* ranged from elliptic to cuneate, with an attenuate base and an acute apex, on average 77.3 ± 34.7 mm (95% CI) long and 24.0 ± 8.3 mm wide (Fig. 2.1B). They were sub-membranaceous, glabrous and entire, borne on a petiole up to 10 mm long. *P. colorata* leaves were also typically elliptic but ranged to ovate; their laminae were coriaceous, glabrous, and entire or undulate, had a cuneate base and an acute to obtuse apex, and were generally larger than *A. pusilla* at 88.5 ± 36.6 mm long and 30.2 ± 12.1 mm wide, with a ≤ 10 mm petiole (Fig. 2.1C). Across the population the two species overlapped in leaf dimensions; 62% of *A. pusilla* and 61% of *P. colorata* leaves were within 1 standard deviation (S.D.) of the other's mean lamina length, and 51% of *A. pusilla* and 29% of *P. colorata* leaves were within 1 SD of the other's mean leaf width. The two species shared similar reflectance spectra for the green as well as for the red portions of the leaf's adaxial surface (Figs. 2.2A and 2.2B), indicating that there was negligible difference in lamina colour.

Fig. 2.2. Reflectance spectra of leaves. Proportions of reflected light from (A) green, and (B) red portions of *Pseudowintera colorata* (broken grey line) and *Alseuosmia pusilla* (solid black line) lamina. Data are the mean $\pm$ SE.

2.4.2 Shape Analysis

The first four principle components of the PCA analysis accounted for 98.0% of the total leaf shape variation. Most (89.6%) of this was attributable to variation in leaf size and to size-associated shape differences represented by PC1 (Fig. 2.3A), with leaves of *A. pusilla* being generally smaller than those of *P. colorata*. In the remaining three PCs, *P. colorata* exhibited the wider variation in leaf shape, as evidenced by a greater spread in SD as compared with *A. pusilla*. PC2 accounted for 4.1% of the total variance in shape; it represented the morphological progression from an asymmetric elliptic for which lamina area was greater at the left- than at the right-hand side of the midrib as viewed from the leaf base on the adaxial surface (abbreviated hereafter as ‘left-biased’), through to an asymmetric ‘right-biased’ cuneate lamina (Fig. 2.3B). PC3 accounted for 3.4% of the variance and represented the opposite range to PC2, lamina shape varying from right-biased elliptic to left-biased cuneate

(Fig. 2.3C). PC4 accounted for 1.0% of the variance, representing shapes from narrow linear to an elliptic (Fig. 2.3D). (For two-dimensional separation of *P. colorata* and *A. pusilla* individuals along PC1-4, see Appendix A.2).

Fig. 2.3. Principal components analysis (PCA) of leaf shape variation in *Pseudowintera colorata* and *Alseuosmia pusilla*. PCA reduced 98.1% of leaf shape variation between the two species into four principal components (PC1–4). For each PC, proportionate frequencies of *P. colorata* (grey bars) and *A. pusilla* (black bars) leaves are given for up to 3 standard deviations (SD) of the mean leaf shape (0). Leaf silhouettes show typical shapes, proportionately scaled, represented by each 1 SD on each PC. (A) PC1, accounted for 89.6% of total leaf shape variation; (B) PC2, 4.1%; (C) PC3, 3.4%; (D) PC4, 1%.

2.4.3 Shape Discrimination

The leaf shapes of *P. colorata* and *A. pusilla* were morphometrically distinct from those of all seven other species growing in their vicinity; 97.3% of *P. colorata* and *A. pusilla* leaves (grouped together) were correctly identified by discriminant analysis. When the analysis was restricted to *P. colorata* and *A. pusilla* only, discriminant analysis incorrectly classified 7.1% of *A. pusilla*, of which most (5.4%) were classified as *P. colorata*. For *P. colorata*, 29.4% were misidentified as *A. pusilla*; none was misidentified as any other species (Table 2.1). Thus, 17.4% of *A. pusilla* and *P. colorata* were misclassified. (For the discriminant analysis of *P. colorata* and *A. pusilla* in the absence of the neighbouring species, see Appendix A.3)

Table 2.1. Percentage of correct identification of species by cross validated discriminant analysis using the leaf margin landmarks.

Original species group membership	Predicted species group membership based on leaf shape (%)								
	<i>L.m</i>	<i>C.c</i>	<i>C.f</i>	<i>C.g</i>	<i>G.l</i>	<i>M.s</i>	<i>P</i>	<i>A.p</i>	<i>P.c</i>
<i>Lophozonia menziesii</i> (<i>L.m</i>)	76.9	19.2	0.0	0.0	0.0	0.0	0.0	3.8	0.0
<i>Coprosma colensoi</i> (<i>C.c</i>)	0.0	96.3	0.0	3.1	0.0	0.0	0.0	0.5	0.0
<i>Coprosma foetidissima</i> (<i>C.f</i>)	0.0	0.0	80.0	6.7	0.0	0.0	0.0	10.0	3.3
<i>Coprosma grandifolia</i> (<i>C.g</i>)	0.0	9.6	1.4	86.3	0.0	0.0	0.0	0.0	2.7
<i>Griselinia littoralis</i> (<i>G.l</i>)	0.0	0.0	0.0	0.0	89.5	5.3	0.0	5.3	0.0
<i>Myrsine salicina</i> (<i>M.s</i>)	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
<i>Prumnopitys</i> spp. (<i>P</i>)	1.4	1.4	0.0	0.0	0.0	0.0	94.4	2.8	0.0
<i>Alseuosmia pusilla</i> (<i>A.p</i>)	0.0	1.3	0.0	0.2	0.0	0.2	0.0	92.9	5.4
<i>Pseudowintera colorata</i> (<i>P.c</i>)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.4	70.6

P. colorata exhibited a more even spread of individuals within ± 3 SD from the mean leaf shape than did *A. pusilla*, especially in the characteristics represented by PC2 to PC4 (Figs. 2.3A-D). *A. pusilla* had a narrower range of shapes characterised by a greater proportion of individuals approaching its mean leaf shape; thus, it was easier for the discriminant analysis to categorise the species correctly, explaining why a greater proportion of *P. colorata* was mistaken as *A. pusilla* rather than the other way round. *A. pusilla* individuals that were found beyond ± 3 SD from their mean leaf shape were closer to the mean shape of *P. colorata* than to their own mean shape (Table 2.1).

2.4.4 Elevational variation in leaf shape

Leaf size and shape represented by PC1 increased similarly in both species at higher elevations (species identity: $r^2 = 0.21$, $P < 0.001$; slopes are parallel: d.f = 1, $F = 0.28$, $P = 0.59$; Fig. 2.4A). Leaves of *P. colorata* were constantly larger across the elevational gradient (intercepts differ: d.f = 1, $F = 170$, $P < 0.001$). However, some features of leaf shape varied with elevation differently between the species (all interactions between species and elevation in PC2, PC3, PC4 were statistically significant; $P < 0.05$). In *A. pusilla* leaf shape shifted from left-biased elliptic to right-biased cuneate, whilst *P. colorata* shifted slightly in the opposite direction (PC2; Fig. 2.4B). For the attributes associated with PC3, the left-biased cuneate laminae of *A. pusilla* became less abundant at higher elevations, replaced by right-biased elliptic leaves; there was no effect in *P. colorata* (Fig. 2.4C). Finally, the narrow linear laminae of *A. pusilla* (PC4) were replaced by more elliptic laminae, while there was no effect in *P. colorata* (Fig. 2.4D).

Fig. 2.4. Leaf shape of *Alseuosmia pusilla* and *Pseudowintera colorata* in relation to elevation. Leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, as illustrated in Fig. 2.3. *A. pusilla* (crosses) and *P. colorata* (circles). Each point represents one leaf from an individual plant. The y-axes extend ± 5 S.D. along each PC from the mean leaf shape, which is represented by 0. Linear regressions show elevational trends in leaf shape variation explained by each PC for *A. pusilla* (black line) and *P. colorata* (grey line). Leaf silhouettes are proportionately scaled and show typical shapes represented by ± 3 S.D. and the mean leaf shape (0).

2.4.5 Shape comparison among neighbours

The probability that leaves of *A. pusilla* would be similar in shape to those of *P. colorata* was greater the closer the two species were to one another (Figs. 2.5A-D). This probability fell rapidly at separation distances greater than about 200 m, irrespective of which criterion was used to describe shape. Similarity in leaf size and shape (represented by PC1) declined the most with increasing separation distance (Fig. 2.5A), followed by characters represented by PC2 (Fig. 2.5B) and then PC3 (Fig. 2.5C). Leaves that varied from narrow linear to elliptic (PC4 characters) were the least likely to change over distance (Fig. 2.5D). Patterns of similarity between *P. colorata* individuals and the surrounding *A. pusilla* did not change along the transect (see Figs. 2.6A-D).

Fig. 2.5. Comparison of individual *Alseuosmia pusilla* principal component (PC) values to the neighbouring *Pseudowintera colorata* population. Log-likelihood scores for similarity in leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, comparing *A. pusilla* individuals to the surrounding *P. colorata* over distance. Decreases in log-likelihood scores indicate reduced similarities in leaf shape represented by the corresponding PC.

Fig. 2.6. Comparison of individual *Pseudowintera colorata* principal component (PC) values to the neighbouring *Alseuosmia pusilla* population. Log-likelihood scores for similarity in leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, comparing *P. colorata* individuals to the surrounding *A. pusilla* over distance. Decreases in log-likelihood scores indicate reduced similarities in leaf shape represented by the corresponding PC.

2.5 DISCUSSION

There are five key findings from this study of spatial variation in leaf shape. (i) The chemically defended *P. colorata* is morphologically distinct from all surrounding heterospecifics except for *A. pusilla* (Table 2.1). (ii) The range of leaf shapes of *P. colorata* overlaps significantly with that of *A. pusilla*, meaning that 17.4% of the leaves of the two species were misclassified (Table 2.1). (iii) *P. colorata* and *A. pusilla* share similar lamina colours (Figs. 2.2A and 2.2B). (iv) Leaf shape in the two species varies similarly across an elevational gradient (Fig. 2.4A-D). (v) Leaves of *A. pusilla* are more likely to resemble those

of *P. colorata* the closer the two species are to one another (Fig. 2.5A-D). These results add weight to the hypothesis that *A. pusilla* could be a Batesian mimic of *P. colorata*.

Batesian mimicry requires that distinguishable traits shared by model and mimic are identifiable by the predator and associated with unpalatability; the more conspicuous the signal, the more readily is this association learned (Cott 1940, Harvey 1983). For leaf shape to be effective as a visual deterrent, it would therefore need to be sufficiently distinct from those of surrounding species. Previous studies of putative Batesian mimics did not always address the question that the shared traits are exclusive to the putative model and mimic. Certain Australian mistletoes, for example, are remarkably similar in leaf colour and shape to those of their hosts (Barlow and Wiens 1977, Ehleringer et al. 1986), yet some of these similarities are also shared with other species in the surrounding plant community (Blick et al. 2012) and so it is likely that herbivores would use different features to discriminate between them (Canyon and Hill 1997). Like the mistletoes, the leaves of our putative model and mimic are very similar in shape and colouration; 17.4% of the leaves were misclassified. However, in contrast to the mistletoes, leaf shapes in our focal species are substantially different from those of all other sympatric trees and shrubs; discriminant analysis correctly classified 97% of *A. pusilla* and *P. colorata* when they were grouped together and compared against the surrounding species. Thus, there is a real possibility that leaf shape *per se* might be used as a visual deterrent in these species.

Leaf shape often changes along environmental gradients (McDonald et al. 2003). Thus, if mimicry is to be effective, both the model and mimic would need to respond similarly to any environmental factor that affects leaf shape. Along the elevational gradient spanned by our transect, changes in leaf size, and in leaf shape related to variation in leaf size, were similar for *P. colorata* and *A. pusilla* (Fig. 2.4A); these were PC1 features, accounting for 90% of the total shape variation shared between the two species (Fig. 2.3A). Moreover, the leaves of *A. pusilla* were morphologically most similar to those of neighbouring *P. colorata* within a radius of around 200m, with a sharp decline in leaf similarity among plants that were further apart (Figs. 2.5A-D). We do not know the degree to which genetic and environmental factors control leaf shape in these species, but it is evident that the two respond very similarly.

A foraging herbivore would most likely encounter potential food sources in succession rather than simultaneously, and would therefore rely on memory to associate leaf morphology with unpalatability. The accuracy of discriminating between successively viewed objects decreases

rapidly due to inefficiencies in coding and retrieving of memories, as shown both in humans (Uchikawa and Ikeda 1981) and honeybees (Dyer and Neumeyer 2005). Thus, effective mimicry does not require that the mimics be exact replicas of their models; a plant would need only to resemble its protected neighbours with sufficient accuracy to account for the level of degraded memory. Rather than a general similarity in leaf morphology, the similarity shared between *A. pusilla* and its immediate *P. colorata* neighbours, which is maintained across the shared growing range, would further reduce a foraging herbivore's ability to discriminate between them.

We do not know which of the potential herbivores may have been important in driving the evolution of leaf morphology in *A. pusilla*. The terrestrial fauna was historically dominated by moa, which were generalist herbivores consuming a wide variety of plants (Wood et al. 2008, 2012), but there is no evidence to suggest that they were the main selective pressure in this system. Little, too, is known about the insect herbivores of *A. pusilla*. Forest populations of *A. pusilla* often show evidence of damage by Lepidopteran larvae, but it is not known whether these and/or other herbivorous invertebrates attack or are deterred by *P. colorata*. Insects have poor spatial acuity compared with larger vertebrates (Giurfa et al. 1996), but once close enough to a plant they may similarly resolve small objects to recognise patterns and shapes (Giurfa and Menzel 1997). Thus, leaves might well have been used not only by moa, but also by insect herbivores for identification purposes.

Unequivocal proof of mimicry requires evidence that: (i) both the mimic and a known model are attacked by the same herbivore; (ii) the herbivore is unable to distinguish between them; and (iii) this confusion increases the fitness of the mimic. To date, no study has satisfied these three criteria in plants (Schaefer and Ruxton 2009, 2011). However, my study provides strong evidence that leaf shape is precisely matched between an undefended plant species and a chemically-defended unrelated species across a shared growing range, and therefore represents an important first step at establishing Batesian mimicry between *A. pusilla* and the putative model, *P. colorata*. I highlight how a spatially-explicit morphometric analysis provides a powerful tool to study visual Batesian mimicry in plants.

Chapter 3: Introduced mammalian herbivores select for increased distinctiveness within an unpalatable species and its purported mimic

3.1 ABSTRACT

Unpalatable prey that warn their predators of their defences, either through conspicuousness or distinctiveness, can share the burden of educating their predators to avoid them if their warning signal is similar. However, warning signals often vary due to trade-offs in associative fitness benefits and costs. Batesian mimics gain protection through copying these signals, and theory predicts that mimics should evolve to be most like their model. Yet Batesian mimics also face trade-offs that influence the accuracy of their signal matching. Unlike in animals, visual conspicuousness and/or distinctiveness to advertise unpalatability in plants has received little attention. Likewise, defensive Batesian mimicry in plants is lacking in empirical evidence. I use population abundance estimates and a geometric morphometric approach to quantify spatial variation in the distinctive leaf shape and size of the chemically defended plant, *Pseudowintera colorata*, between two populations that are either exposed to high or low numbers of mammalian herbivores. I compare how abundance, and leaf shape and size varies to a sympatric, unrelated, putative Batesian mimic, *Alseuosmia pusilla*. I found that when exposed to higher levels of mammalian herbivores, unlike other sympatric tree and shrub species, the distinctive leaf shape of both *P. colorata* and *A. pusilla* reduced in variation. The leaf shape of both species also responded in a similar way at a closer magnitude. Both *P. colorata* and *A. pusilla* became more abundant, however, they remained at a similar proportion to each other and at a similar level of morphological similarity between sites. Lastly, *A. pusilla* was found growing three times closer in distance to *P. colorata* individuals when exposed to more mammalian herbivores. The data are consistent with Batesian mimicry, wherein increased herbivory pressure selects for a stronger signal in the distinctive characteristic of the defended plant, and through the selection for mimicry, variation in the mimic's phenotype converges on the model's phenotype. Additionally, when alternative palatable prey are preferentially targeted, *P. colorata* increased in abundance and *A. pusilla* followed suit proportionately. My study provides the first quantitative evidence of variation in leaf shape between populations of high and low levels of mammalian herbivores. This provides further evidence for the hypothesis that *A. pusilla* is a Batesian mimic of *P. colorata*.

3.2 INTRODUCTION

It is well recognised that prey that invest significantly in defence against predators generally advertise this defence through conspicuous signals (Wallace 1867, Darwin 1871, Poulton 1890, Cott 1940). However, defended prey that are non-conspicuous or even cryptic may also be avoided as long as they are distinct from other potential prey (Sherratt and Beatty 2003, Merilaita and Ruxton 2007). Uniformity in warning signals increases the probability that predators would learn to avoid the defended prey (Ruxton et al. 2004). Undefended species may evolve to copy this defence signal, taking advantage of a shared predator's learned avoidance via Batesian mimicry (Bates 1862). Generally, the more conspicuous and/or distinctive the signal is, the more readily the association is learned (Cott 1940, Harvey 1983).

Yet variation in the strength of warning signals exists when there are trade-offs in associative fitness benefits and costs. These trade-offs may come as a response to variation in environmental pressures (Hazel 2002, Sword 2002, Ojala et al. 2007, Williams 2007, Lindstedt et al. 2008a, 2008b); in simple environments less signal variation might be expected due to fewer interactions requiring trade-offs, but in complex environments with more interactions the signal variation would be greater (Lindstedt et al. 2011). Variation in signals may also be affected by the level of risk of predation. Stronger signals may be favoured when the risk of predation is high in order to reduce predator mistakes, e.g. when density of prey is low relative to predator abundance (Summers et al. 2015). Batesian mimics also face trade-offs, which can affect the degree to which they match their model's signal. In theory a mimic should evolve to be most like its model (Ruxton et al. 2004), but often mimics resemble their models less precisely than expected (Sherratt 2002). This can be due to the number of alternative models present (Edmunds 2000, Sherratt 2002), the type and diversity of predators (Pekár et al. 2011), the relatedness of surrounding mimics (Johnstone 2002, Penney et al. 2012), the undesirable scrutinisation that exaggerated conspicuousness may bring to a mimic (Speed and Ruxton 2010), the relative abundance of mimics to models (Sherratt 2002, Penney et al. 2012) and the availability of alternative prey (Holling 1965, Getty 1985), or the extra competition between mimics and their models a similar phenotype may produce (Pfennig and Kikuchi 2012).

Leaf shape and size have long been hypothesised to play a role in deterring herbivores (Brown and Lawton 1991). Although leaf morphology assists with regulation and mitigation of the effects of environmental stressors such as shoot temperatures (Hegazy and El Amry

1998, Roth-Nebelsick 2001), light interception (Horn 1971, Tsukaya 2005), avoidance of wind damage (Anten et al. 2010), salt exclusion (Sinclair and Hoffmann 2003), drought avoidance, or nutrient deficiencies (Cunningham et al. 1999, Leigh et al. 2011), the diversity of leaf shape and size in plants suggests that it is not exclusively functionally dependent on any one ecological strategy (Nicotra et al. 2011). Leaf shape has, however, been demonstrated to influence the consumption, performance, and ovipositioning choices of insect herbivores (Rausher 1978, Mackay and Jones 1989, Rivero-Lynch et al. 1996, Campitelli et al. 2008a). It can also provide a visual cue of a plant's palatability (Dell'aglio et al. 2016), and therefore leaf shape may act as a distinctive signal for herbivore recognition of palatability.

Unlike in animals, visual conspicuousness and/or distinctiveness to advertise unpalatability in plants has received little attention. Conspicuousness in the form of aposematic colouration may advertise the presence of thorns (Lev-Yadun 2001, Rubino and McCarthy 2004), or defensive compounds (Cooney et al. 2012), and in autumnal leaves may display low nutrient quality and/or defensive investment (Hamilton and Brown 2001, Archetti and Brown 2004). Even less attention has been given to the evolution of Batesian mimics in plants. In some reported cases, a resemblance may occur only in the presence of a particular species. For example the leaves of the *Boquila trifoliolata* vine are morphologically similar to those on the particular supporting host tree (Gianoli and Carrasco-Urra 2014), and the leaf form and overall habit of *Iris atrofusca* resemble those of the unpalatable *Asphodelus ramosus* (Shimshi 1979). In other studies the purported mimetic features may not actually be a distinctive characteristic within the environment. Leaves of some Australian mistletoes closely resemble those of their host plant (Barlow and Wiens 1977, Ehleringer et al. 1986), but these similarities are shared with other species in the surrounding plant community (Blick et al. 2012), therefore leaf shape in this case is unlikely to be used by herbivores to discriminate between species (Canyon and Hill 1997).

Warning signal similarity, both within and between species, is assumed to increase the efficacy of warning signals (Müller 1879, Beatty et al. 2004). Therefore the costs of educating naïve predators to avoid the warning signal are shared across individuals with similar signals. Because of this positive frequency dependent selection for signal design, effective predator learning should select against dissimilar signals, favouring uniformity (Müller 1879, Beatty et al. 2004, Rowland et al. 2007). Likewise, increased predatory pressures increase the strength of a signal, i.e. by reducing the amount of variation and

selecting for uniformity (Lindström et al. 1999, 2001a, Sherratt and Beatty 2003). For a conspicuous and/or distinctive leaf shape to be considered a mimetic signal, we would expect that (i) herbivory pressure reduces the variation in leaf shape of unpalatable models, thereby producing a stronger signal, and (ii) through selection for mimicry, variation in the mimic's leaf shape will converge on the model's phenotype. Further, to test the benefits of this convergence of leaf shape under increased herbivory pressure where alternative palatable species are preferentially targeted, two additional patterns would likely emerge: (iii) the model would increase in abundance and (iv) the mimic would follow suit proportionately. In this study, the four predictions above were tested to evaluate the hypothesis that an unpalatable plant species with a distinct leaf shape may be mimicked by a palatable species to avoid herbivore detection.

These hypotheses were evaluated in a purported mimicry system between *P. colorata* and its putative Batesian mimic, *A. pusilla* under herbivory pressure from introduced mammals. The impact of introduced mammalian herbivores in New Zealand has received a lot of attention, is well studied, and there exist areas of forest with controlled and uncontrolled populations. In the presence of uncontrolled populations of deer, goats, and possums, palatable species are successively removed, reducing the forest understorey density and increasing the abundance of unpalatable species (Allen et al. 1984, Husheer et al. 2003). The leaves of *P. colorata* are especially unpalatable to introduced mammalian herbivores (Forsyth et al. 2005) and it often becomes the most dominant understorey tree species (Allen et al. 1984, Husheer et al. 2003). The leaves of *P. colorata* are rich in polygodial, a sesquiterpene dialdehyde that imparts a pungent taste and is a potent insect antifeedant (Asakawa et al. 1988). Leaves of *P. colorata* may have similarly been avoided by the extinct flightless birds known as moa (Greenwood and Atkinson 1977) that are thought to have presented historically significant browsing pressures (Atkinson and Greenwood 1989, Wood et al. 2013). In contrast, the leaves of *A. pusilla* contain few anti-herbivory compounds (Cambie and Parnell 1970) and are readily eaten by introduced ungulates (Greenwood and Atkinson 1977, Nugent et al. 2001). Where both species occur together they share a leaf appearance that is distinct from the surrounding species, they respond similarly in leaf shape across an environmental gradient, and the leaves of *A. pusilla* are more likely to resemble those of *P. colorata* the closer the two species are to one another (Yager et al. 2016). For this study, variation in leaf shape of *P. colorata* and *A. pusilla* was compared between two field sites: one with a low abundance of introduced mammalian herbivores, and one with a high abundance of mammalian herbivores (Fig. 3.1). I

hypothesise that at the high mammalian herbivore site: (i) variation in leaf shape will be reduced in the unpalatable *P. colorata* but not in the surrounding sympatric species such that its morphology is acting as a stronger signal of its unpalatability; (ii) variation in leaf shape of the undefended *A. pusilla* will also decrease; (iii) variation in *P. colorata* leaf shape across an environmental gradient will not only be closely matched by variation in foliar morphology of *A. pusilla*, but also be of a comparable magnitude; (iv) *A. pusilla* will be similar in leaf shape to the population of *P. colorata* rather than when they grow close to one another; (v) *A. pusilla* will increase in abundance in proportion to an increase in *P. colorata*; and (vi) the degree of similarity between *A. pusilla* and *P. colorata* will be the same as that of the low herbivore site.

Fig. 3.1. Location of field sites within the south-western portion of the Tararua Forest Park, near Wellington, New Zealand. (A) Kapakapanui field site; “High Herbivore”, and (B) Otaki Forks field site; “Low Herbivore”. Project Kākā pest control area denoted by red shading. Scale bar = 4 km. Map reproduced from Department of Conservation (2014).

3.3 MATERIALS AND METHODS

3.3.1 Study Sites

This study compared *A. pusilla* and *P. colorata* across two sites; one with low numbers of herbivores, and one with high numbers of introduced herbivores. The Otaki Forks area (Low Herbivore site, hereafter abbreviated to LH) has received aerial poison drops targeting possums since 1996 (six-yearly aerial bait drop programme until 2010 when it became three-yearly). The Kapakapanui area (High Herbivore site, hereafter abbreviated to HH) has received no Department of Conservation intervention in pest animal management (ungulates, possums, etc.) (Fig. 3.1). This is the only such site in the Tararua ranges and is used as a study site to compare the effectiveness of pest control programmes (Urlich and Brady 2003). Due to pest herbivore management, Otaki Forks has substantially fewer deer, goats and possums than the Kapakapanui site as measured by counts of faecal pellets and waxtag Bite Mark Index monitoring (Fig. 3.2) (Department of Conservation 2014).

Fig. 3.2. Changes in estimated population densities of ungulates (deer and goat) (A), and possums (B), between Otaki Forks; “Low Herbivore” field site (grey line), and Kapakapanui; “High Herbivore” field site (black line). Changes in ungulate densities are estimated by a faecal pellet index across 40 transects randomly located in the Project Kākā pest control area (red shaded area in Fig. 3.1) and non-treatment areas (area outside red shaded area in Fig. 3.1). Possum densities are estimated by the average proportion of waxtags bitten across two lines of 20 waxtags in the Project Kākā pest control area and non-treatment area. Data are the mean $\pm$ 95% C.I. Data reproduced from Department of Conservation (2014).

3.3.2 Plant Material

Plant material collection and sampling technique for the LH site was the same as described in section 2.3.1 of Chapter 2.

For the HH site, *P. colorata* and *A. pusilla* leaves were collected from a 3.5km stretch of track (S40 55.15, E175 08.14 to S40 54.66, E175 09.01) during January 2013 through montane forest in the Kapakapanui region of the Tararua Forest Park, New Zealand (see Appendix B.1). The collection area covered the elevational range of 517 – 715 m, sharing a common suite of plant species and like the Otaki Forks collection, was characterised by a shift from the lower conifer-broadleaf forest to the higher *Lophozonia*- and *Fuscospora*-dominated forest. Plants sampled from the Kapakapanui and Otaki Forks transects were from similar slope aspects. Unlike Otaki Forks, *A. pusilla* and *P. colorata* grew along the entire transect, and *P. colorata* was the prominent understorey tree along the final 2.5 km of the transect. Every *P. colorata* and *A. pusilla* within 2 m of one side of the track was sampled if the plants were between 15 cm and 2 m tall, and their location recorded using a Garmin 60 GPS (Olathe, USA; accuracy: ± 2 -10 m). The youngest fully expanded leaf was excised from a total of 244 *P. colorata* and 322 *A. pusilla* plants. When there were multiple branches, the youngest fully expanded leaf was removed from the highest branch. All leaves were refrigerated within 6 hours of collection.

In addition, leaves from seven other sympatric tree and shrub species (*Myrsine salicina*, *Griselinia littoralis*, *Lophozonia menziesii*, *Coprosma grandifolia*, *C. foetidissima*, *C. colensoi*, and *Prumnopitys ferruginea*) were collected for which leaf size and shape were similar to *A. pusilla* and *P. colorata*, and which were located within 1.5m of *P. colorata* and *A. pusilla* plants. Between 25 and 60 individuals from each species were sampled; one leaf was collected from each individual from the closest branch facing the nearest *P. colorata* or *A. pusilla* plant. All leaves were refrigerated within 6 h of collection.

Plant material collected was approved under Part IIIB of the Conservation Act 1987 and Section 49 of the Reserves Act 1977 with the New Zealand Department of Conservation permit number WE/34243/FLO.

3.3.3 Herbivory, abundance and neighbour proximity estimation

The relative abundance of *A. pusilla* and *P. colorata* between both sites was estimated by counting the number of *A. pusilla* and *P. colorata* within 30 cm either side along a 20 m

transect. A total of 10 transects were implemented (five at each site) with the start point at least 5 m from either side of the walking track and direction randomly assigned. Transects were located between 650 – 710 m elevation at each site.

To estimate the proximity of surrounding species to *P. colorata* individuals, 10 relatively isolated *P. colorata* between 0.5 m and 2 m tall were randomly selected at both sites. The distance of seven sympatric tree and shrub species individuals common to both sites (see section 3.3.2 of this chapter for species names) as well as *A. pusilla*, was recorded from each focal *P. colorata*, up to a 4 m radius.

Assumed invertebrate herbivore damage to *A. pusilla* and *P. colorata* leaves was quantified at locations where the proportions of numbers of individuals of *A. pusilla* to *P. colorata* were 0, 0.25, 0.50, 0.75, and 1. A visual estimation of the proportion of removed lamina per plant was performed on individuals within 2 m radius plots. Twenty plots were located between 650 – 710 m elevation at each site and were selected based on the existing proportion of *A. pusilla* to *P. colorata* in the area.

3.3.4 Leaf Shape Capturing

Digital images of leaves were captured using the same equipment, software and techniques as described in section 2.3.2 in Chapter 2.

3.3.5 Statistical Analysis

To compare variation in leaf shape of *A. pusilla* and *P. colorata* within each site, statistical analysis techniques were the same as described in section 2.3.4 in Chapter 2.

To compare variation in leaf shape of *A. pusilla* and *P. colorata* between the two sites, a principal component analysis (PCA) was run using the partial Procrustes coordinates of all samples from both sites. The 70 partial Procrustes coordinates were reduced to four principle components (PC) that explained the majority of shape variation for each leaf across both sites. A PCA was also run on the partial Procrustes coordinates of the seven sympatric species to compare changes in shape variation between sites. In order to test if the amount of shape variation differed between the two sites, a Levene's Test was performed to test the overall homogeneity of variance across the four PC's for *A. pusilla* and *P. colorata*, and the seven other species collected. Linear regressions for each PC as a function of elevation were compared for each species by ANCOVA. All other comparisons of shape variation where data had to be transformed were made with data independent of site. To test differences

between the means of the amount of leaf damage, and the proximity to *P. colorata* between sites, as the normality of data was not met a Wilcoxon rank sum test was used.

3.4 RESULTS

3.4.1 Leaf Morphology

From the HH site, the leaf laminae of *A. pusilla* ranged from elliptic to cuneate, with an attenuate base and an acute apex, on average 88.4 ± 1.4 mm (95% C.I.) long and 24.3 ± 0.4 mm wide (Fig. 3.3). They were sub-membranaceous, glabrous and entire, borne on a petiole up to 10 mm long. *P. colorata* leaves were also typically elliptic but ranged to ovate; their laminae were coriaceous, glabrous, and entire or undulate, had a cuneate base and an acute to obtuse apex, and were generally larger than *A. pusilla* at 91.9 ± 2.0 mm long and 30.3 ± 0.7 mm wide, with a ≤ 10 mm petiole (Fig. 3.3). Across the population the two species overlapped in leaf dimensions; 69% of *A. pusilla* and 59% of *P. colorata* leaves were within 1 standard deviation (S.D.) of the other's mean lamina length, and 38% of *A. pusilla* and 29% of *P. colorata* leaves were within 1 S.D. of the other's mean leaf width.

Fig. 3.3. Selected examples to show the range of leaf morphology across the elevational gradient of (A) *Alseuosmia pusilla* and (B) *Pseudowintera colorata* from Kapakapanui, “High Herbivore” site, and (C) *A. pusilla*, and (D) *P. colorata* from Otaki Forks, “Low Herbivore” site. Scale bar = 20 mm.

For leaf morphology data from the LH site, refer to section 2.4.1 in Chapter 2.

3.4.2 Shape Analysis

Using the combined leaves from the HH and LH sites to compare leaf shape variation, the first four principle components of the PCA accounted for 97.7% of the total leaf shape variation. Most (87.6%) of this was attributable to variation in leaf size and to size-associated shape differences represented by PC1 (Fig. 3.4A), with leaves of *A. pusilla* being generally smaller than those of *P. colorata*. In the remaining three PC's, *P. colorata* at both sites exhibited the wider variation in leaf shape compared with *A. pusilla* as evidenced by a greater spread in S.D. PC2 accounted for 4.7% of the total variance in shape; it represented the morphological progression from an elliptic lamina through to asymmetric cuneate for which the lamina area was greater at the right- than the left-hand side of the midrib as viewed from the leaf base on the adaxial surface (abbreviated hereafter as 'right-biased') (Fig. 3.4B). PC3 accounted for 4.3% of the variance and represented the lamina shape varying from right-biased elliptic with a narrow petiole to left-biased elliptic with a narrow petiole (Fig. 3.4C). PC4 accounted for 1.1% of the variance and represented the opposite range to PC3, lamina shape varying from left-biased elliptic with a thicker petiole to right-biased elliptic with a thicker petiole (Fig. 3.4D).

Fig. 3.4. Principal components analysis (PCA) of leaf shape variation in *Pseudowintera colorata* and *Alseuosmia pusilla*. PCA reduced 97.7% of leaf shape variation between the two species into four principal components (PC1-4). For each PC, proportionate frequencies of *P. colorata* (High Herbivore = grey horizontal lined bars, Low Herbivore = black hash bars) and *A. pusilla* (High Herbivore = solid grey bars, Low Herbivore = solid black bars) leaves are given for up to 3 standard deviations (S.D.) of the mean leaf shape (0). Leaf silhouettes show typical shapes, proportionately scaled, represented by each 1 S.D. on each PC. (A) PC1, accounted for 87.6% of total leaf shape variation; (B) PC2, 4.7%; (C) PC3, 4.3%; (D) PC4, 1.1%.

3.4.3 Leaf shape variation between sites

The Levene's Test indicated that *P. colorata* and *A. pusilla* at the HH site has less leaf shape variation as described by PC1 and PC2 (equalling 92.3% of the total variation in leaf shape) when compared with the LH site (Fig. 3.4A-B, Table 3.1). Of the seven other species growing in the vicinity of *P. colorata* and *A. pusilla* (see section 3.3.2 of this chapter for species names), leaf shape variation was comparable at both sites for all but 8.2% of the total shape variation represented by PC2 (Table 3.1). (For two-dimensional separation of *P. colorata* and *A. pusilla* individuals along PC1-4 within and between the two sites, see Appendix B.2).

Table 3.1. Levene's test of equal variances between the High Herbivore (HH) and Low Herbivore (LH) sites for the four principal components (PC) representing 97.7% of the total shape variation between *Alseuosmia pusilla* and *Pseudowintera colorata*, and 96.3% between seven sympatric tree and shrub species common to both sites (PC1: 77.8%, PC2: 8.2%, PC3, 6.9%, PC4: 3.4%) - see section 3.3.2 of Chapter 3 for species names. Significant values indicate differences in the amount of leaf shape variation explained by each PC between the HH and LH sites.

Levene's Test of Equal Variances between sites		
	Between <i>A. pusilla</i> and <i>P. colorata</i>	Between other sympatric species
PC1	F(1,1242) = 33.28, p < 0.01	F(1,214) = 3.53, p = 0.06
PC2	F(1,1242) = 8.87, p < 0.01	F(1,214) = 11.14, p < 0.01
PC3	F(1,1242) = 0.01, p = 0.93	F(1,214) = 0.62, p = 0.43
PC4	F(1,1242) < 0.01, p = 0.98	F(1,214) = 3.74, p = 0.06

3.4.4 Elevational variation in leaf shape within sites

The attributes of leaf size and of leaf shape related to leaf size represented by PC1 increased similarly in both species at higher elevations at the LH site (Table 3.2A-B) with leaves of *P. colorata* constantly larger across the elevational gradient (Table 3.2C). At the HH site, leaf shape represented by PC1 slightly decreased similarly in both species (Table 3.2A-B) but there was no difference between leaves of *P. colorata* and *A. pusilla* across the elevational gradient (Table 3.2C; Fig. 3.5A). PC2 represented leaf width and remained constant in both species across higher elevations at the LH site (Table 3.2A-B) with the leaves of *P. colorata* consistently wider (Table 3.2C). At the HH site, *A. pusilla* leaves became slightly narrower at

higher elevations whilst *P. colorata* leaf width remained relatively stable (Table 3.2A; Fig. 3.5B). For the attributes associated with PC3, at the LH site there was no discernible change in petiole diameter, although the asymmetry of the lamina changed in *P. colorata* from a left-biased elliptic laminae to a right-biased elliptic laminae at higher elevations; there was no effect in *A. pusilla* (Table 3.2A). At the HH site, both species responded similarly, from a right-biased elliptic laminae to a left-biased elliptic laminae at higher elevations (Table 3.2A-B) but with no difference between leaves of *P. colorata* and *A. pusilla* across the elevational gradient (Table 3.2C; Fig. 3.5C). Finally, for the attributes associated with PC4, at the LH site the right-biased elliptic laminae of *P. colorata* changed to a left-biased elliptic laminae at higher elevations; again with no effect in *A. pusilla* (Table 3.2C). At the HH site, both species responded similarly with the left-biased elliptic laminae changing to a right-biased elliptic laminae at higher elevations (Table 3.2A-B), again with no difference between *P. colorata* and *A. pusilla* across the elevational gradient (Table 3.2C; Fig. 3.5D).

Table 3.2. Linear regressions for each principal component (PC) as a function of elevation compared for each species by ANCOVA. (A) The two species response to elevation is similar if the slopes are parallel ($p > 0.05$) on the (B) combined linear regression of species. (C) The intercepts between the two species do not differ if $p > 0.05$, therefore the magnitude of response over elevation is closer between species than when intercepts are different ($p < 0.05$).

Testing significance of:		Low Herbivore Site	High Herbivore Site
(A) Slopes parallel	PC1	F(1,674) = 1.23, $p = 0.27$	F(1,563) = 2.57, $p = 0.11$
	PC2	F(1,674) = 0.63, $p = 0.43$	F(1,563) = 12.93, $p < 0.01$
	PC3	F(1,674) = 14.05, $p < 0.01$	F(1,563) = 0.94, $p = 0.33$
	PC4	F(1,674) = 7.81, $p < 0.01$	F(1,563) = 1.29, $p = 0.26$
(B) Species identity	PC1	$r^2 = 0.02$, $p < 0.01$	$r^2 = 0.01$, $p = 0.01$
	PC2	$r^2 = 0.13$, $p < 0.01$	$r^2 = 0.04$, $p < 0.01$
	PC3	$r^2 = 0.04$, $p < 0.01$	$r^2 = 0.04$, $p < 0.01$
	PC4	$r^2 = 0.03$, $p < 0.01$	$r^2 = 0.02$, $p = 0.03$
(C) Slopes intercept	PC1	F(1,675) = 6.66, $p = 0.01$	F(1,564) = 2.71, $p = 0.10$
	PC2	F(1,675) = 12.06, $p < 0.01$	F(1,564) = 4.67, $p = 0.03$
	PC3	F(1,675) = 2.57, $p = 0.11$	F(1,564) = 2.98, $p = 0.09$
	PC4	F(1,675) = 0.12, $p = 0.73$	F(1,564) = 0.38, $p = 0.54$

Fig. 3.5. Leaf shape of *Alseuosmia pusilla* and *Pseudowintera colorata* in relation to elevation. Leaf shape represented by (A) PC1, (B) PC2, (C) PC3, and (D) PC4, as illustrated in Fig. 3.4. *A. pusilla* (High Herbivore = grey circles, Low Herbivore = black circles) and *P. colorata* (High Herbivore = grey crosses, Low Herbivore = black crosses). Each point represents one leaf from an individual plant. The y-axes extend ± 7 S.D. along each PC from the mean leaf shape, which is represented by 0. Linear regressions show elevational trends in leaf shape variation explained by each PC for *A. pusilla* (High Herbivore = grey line, Low Herbivore = black line) and *P. colorata* (High Herbivore = dashed grey line, Low Herbivore = black dashed line). Leaf silhouettes are proportionately scaled and show typical shapes represented by ± 3 S.D. and the mean leaf shape (0).

3.4.5 Shape comparison among neighbours

A PCA was run using transformed HH site data only to ensure shape variation differences between sites were kept separate and neighbouring plants within each site could be compared to one another. The first four PC's now accounted for 96.7% of the total leaf shape variation; PC1 accounted for 82.5%, PC2 for 7.4%, PC3 for 5.4%, and PC4 for 1.4%. As with the LH site population (refer to section 2.4.5 in Chapter 2), *A. pusilla* individuals at the H.H. site tended to be more similar in leaf shape to that of *P. colorata* the closer the two species were to one another. The probability of similarity of leaf size and associated shape (represented by PC1) declined sharply at a separation distance of about 160 m (Fig. 3.6A). However, leaf width, and shape associated with leaf width (represented by PC2) showed the opposite trend, sharply increasing in similarity over increasing separation distance (Fig. 3.6B). *A. pusilla* showed a weak similarity with surrounding *P. colorata* for leaf shape parameters associated with PC3 and PC4 (Figs. 3.6C-D). Patterns of similarity between *P. colorata* individuals and the surrounding *A. pusilla* either did not change, or showed only a weak pattern along the transect for PC1 – PC3 (see Figs. 3.7A-C). *P. colorata* individuals showed a strong pattern of similarity with surrounding *A. pusilla* at increasing separation distances with leaf shape associated with PC4 (Fig. 3.7D).

Fig. 3.6. Comparison of individual *Alseuosmia pusilla* principal component (PC) values to the neighbouring *Pseudowintera colorata* population at the High Herbivore site. The first four PC's account for 96.7% of the total leaf shape variation. Log-likelihood scores for similarity in leaf shape represented by (A) PC1 (82.5%), (B) PC2 (7.4%), (C) PC3 (5.4%), and (D) PC4 (1.4%), comparing *A. pusilla* individuals to the surrounding *P. colorata* over distance. Decreases in log-likelihood scores indicate reduced similarities in leaf shape represented by the corresponding PC.

Fig. 3.7. Comparison of individual *Pseudowintera colorata* principal component (PC) values to the neighbouring *Alseuosmia pusilla* population at the High Herbivore site. The first four PC's account for 96.7% of the total leaf shape variation. Log-likelihood scores for similarity in leaf shape represented by (A) PC1 (82.5%), (B) PC2 (7.4%), (C) PC3 (5.4%), and (D) PC4 (1.4%), comparing *P. colorata* individuals to the surrounding *A. pusilla* over distance. Decreases in log-likelihood scores indicate reduced similarities in leaf shape represented by the corresponding PC.

3.4.6 Shape Discrimination

Of the HH population, discriminant analysis incorrectly classified 10.2% of *A. pusilla* as *P. colorata*, and 22.5% of *P. colorata* as *A. pusilla*. Of the LH population, 6% of *A. pusilla* was incorrectly classified as *P. colorata*, and 29.9% of *P. colorata* as *A. pusilla*. (Table 3.3). Thus, 16.4% of *A. pusilla* and *P. colorata* from the HH population and 18% from the LH population were misclassified from each other.

Table 3.3. Percentages of correct identification of *Alseuosmia pusilla* and *Pseudowintera colorata* by cross validated discriminant analysis using the leaf margin landmarks at the High and Low Herbivore sites.

Field Site	Original species group membership	Predicted species group membership based on leaf shape (%)	
		<i>A.p</i>	<i>P.c</i>
Otaki Forks (Low Herbivore)	<i>Alseuosmia pusilla</i> (<i>A.p</i>)	94.0%	6.0%
	<i>Pseudowintera colorata</i> (<i>P.c</i>)	29.9%	70.1%
Kapakapanui (High Herbivore)	<i>Alseuosmia pusilla</i> (<i>A.p</i>)	89.8%	10.2%
	<i>Pseudowintera colorata</i> (<i>P.c</i>)	22.5%	77.5%

P. colorata at both sites exhibited a more even spread of individuals within ± 3 S.D. from the mean leaf shape than did *A. pusilla* (Figs. 3.4A-D). *A. pusilla* at both sites had a narrower range of shapes characterised by a greater proportion of individuals approaching its mean leaf shape; thus, it was easier for the discriminant analysis to categorise the species correctly, explaining why a greater proportion of *P. colorata* was mistaken as *A. pusilla* at both sites rather than the other way round. *A. pusilla* individuals that were found beyond ± 3 S.D. from their mean leaf shape were closer to the mean shape of *P. colorata* than to their own mean shape. *A. pusilla* at the HH site were more commonly found beyond ± 3 S.D. from their mean leaf shape than *A. pusilla* from the LH site (Table 3.3).

3.4.7 Species abundance between sites

Both *P. colorata* and *A. pusilla* were more abundant at the HH site compared with LH site with 43% more of the unpalatable *P. colorata* (LH site: $n = 41$, HH site: $n = 96$) and 48% more *A. pusilla* (LH site: $n = 10$, HH site: $n = 21$). However, *A. pusilla* grew closer to *P. colorata* individuals at the HH site than at the LH site (Wilcoxon rank sum $W = 112$, $p < 0.01$). There was no difference between the HH and LH site for the other neighbouring tree and shrub species and *P. colorata*. The median distance *A. pusilla* grew from a *P. colorata* individual was 32 cm in the HH site, with 75% of *A. pusilla* within 51 cm of a *P. colorata*. At the LH site the median distance *A. pusilla* grew from a *P. colorata* individual was 115 cm, with 75% within 185 cm (Fig. 3.8). There was no statistically significant difference in lamina damage between the sites at the different proportions of *A. pusilla* to *P. colorata* (Fig. 3.9).

Fig. 3.8. Box-and-whiskers plots of the proximity to *Pseudowintera colorata* individuals of eight tree and shrub species growing at Otaki Forks “Low Herbivore site” (LH) (light boxes) and Kapakapanui “High Herbivore site”(HH) (grey boxes) including *Alseuosmia pusilla* (*A.p*). Tree and shrub species common to both sites and sympatric to *A. pusilla* and *P. colorata*. Tree and shrub species were: *M.s* – *Myrsine salicina* ($n_{LH} = 30$, $n_{HH} = 15$), *G.l* – *Griselinia littoralis* ($n_{LH} = 6$, $n_{HH} = 4$), *L.m* – *Lophozonia menziesii* ($n_{LH} = 4$, $n_{HH} = 5$), *C.g* – *Coprosma grandifolia* ($n_{LH} = 6$, $n_{HH} = 4$), *C.f* – *C. foetidissima* ($n_{LH} = 9$, $n_{HH} = 7$), *C.c* – *C. colensoi* ($n_{LH} = 29$, $n_{HH} = 51$), *P.f* – *Prumnopitys ferrugine* ($n_{LH} = 3$, $n_{HH} = 5$), *A.p* – *A. pusilla* ($n_{LH} = 18$, $n_{HH} = 39$). Asterisks indicates statistical significance between pairs (Wilcoxon rank sum $W = 112$, $p < 0.01$).

Fig. 3.9. Visually estimated area (%) of leaf lamina removed by herbivory for *Pseudowintera colorata* (*P.c*) and *Alseuosmia pusilla* (*A.p*) at Otaki Forks “Low Herbivore site” (LH) (dark bars), and Kapakapanui “High Herbivore site” (HH) (light bars) from plots that contained different proportions of *A. pusilla* to *P. colorata*: 0 ($n_{LH} = 17$, $n_{HH} = 29$), 0.25 ($n_{LH} = 54$, $n_{HH} = 93$), 0.50 ($n_{LH} = 48$, $n_{HH} = 35$), 0.75 ($n_{LH} = 39$, $n_{HH} = 22$), and 1 ($n_{LH} = 17$, $n_{HH} = 7$). 20 plots at a 2 m radius were used in both the LH and HH sites and were located between 650 – 710 m elevation. There were no statistically significant differences between pairs. Data are means $\pm$ 95% C.I.

3.5 DISCUSSION

There were seven key findings from this study of variation in leaf shape under high and low herbivory pressure. (i) For both species, leaf shape was substantially less variable at the HH site (Fig. 3.4A-B), but only slightly lower for surrounding sympatric species (Table 3.1). (ii) Although leaf shape in the two species varied similarly across an elevational gradient at both sites, the magnitude of the shape changes was only comparable for the two species at the HH site (Table 3.2; Fig. 3.5A-D). (iii) At the HH site *A. pusilla* individuals are more similar in leaf shape to the population of *P. colorata* at large than are those at the LH site (Fig. 3.6A-D). (iv) Both *P. colorata* and *A. pusilla* were more abundant in the presence of high numbers of mammalian herbivores, but a similar proportion of *A. pusilla* to *P. colorata* was present between the two sites. (v) A similar percentage of leaves of the two species were misclassified at the HH and the LH sites, meaning the degree of accuracy in matching of leaf shape between *A. pusilla* and *P. colorata* is similar at each site (Table 3.3). (vi) At the HH

site where alternative palatable species were less abundant, *A. pusilla* was found growing three times closer to *P. colorata* individuals than at the LH site (Fig. 3.8). (vii) Portions of removed laminae for both *A. pusilla* and *P. colorata* were the same between the two sites suggestive that invertebrate herbivory was similar. These results demonstrate that the palatable *A. pusilla* benefits by growing alongside the unpalatable *P. colorata* in the presence of introduced mammalian herbivores. In the presence of increased mammalian herbivores, leaf shape variation is reduced in *P. colorata* and *A. pusilla* and may be acting as a distinctive signal of unpalatability. They add weight to the hypothesis that *A. pusilla* is a Batesian mimic of *P. colorata*, and that introduced mammalian herbivores might effectively be maintaining this historical evolutionary condition.

Within the forests of New Zealand, uncontrolled populations of mammalian herbivores reduce the abundance of palatable species and reduce the density of the forest understorey by removing species that compete with *P. colorata* for resources (Allen et al. 1984, Husheer et al. 2003). This reduction in diversity of species and opening up of the understorey in effect reduces the complexity of the environment. As stronger defensive signals in unpalatable species are promoted by less complex environments (Lindstedt et al. 2011), as well as increased predatory pressures (Lindström et al. 1999, 2001a, Sherratt and Beatty 2003) such as increased herbivore abundances, it can be expected that if leaf shape were acting as a signal there would be less variation in leaf shape at the HH site. In the presence of more mammalian herbivores in this study, variation in leaf shape of both *P. colorata* and *A. pusilla* was lower than that at the LH site; these leaf shape features accounting for 92% of the total leaf shape variation represented by PC1 and PC2. The effect of increased herbivory pressure on leaf shape variation on other species was not shared to the same degree, for which the features accounting for only 8% of the total leaf shape variation (PC2) were reduced (Table 3.1).

Abiotic factors can also influence phenotypic variation in traits that are used as conspicuous and/or distinctive signals (Lindstedt et al. 2008a, 2008b). In plants, leaf shape often changes along environmental gradients (McDonald et al. 2003), therefore in order for leaf shape to be effective as a mimetic signal, leaf shape of the mimic would need to respond similarly to that of its model to influencing environmental factors (Yager et al. 2016). The extent to which leaf shape varied across the elevational gradient was similar for the LH (92%) and HH (93%) sites. However, the magnitude of shape change was similar for *A. pusilla* and *P. colorata* at the HH site (Table 3.2C; Fig. 3.5A, C-D) as represented by PCs 1, 3 and 4 (Fig. 3.4A, C-D).

In contrast, at the LH site, *P. colorata* tended to be larger and wider than *A. pusilla* across the elevational gradient (Fig. 3.5A-D).

At the LH site *A. pusilla* individuals were likely to appear most similar to neighbouring *P. colorata* than to those growing further away (Yager et al. 2016). Because memory decreases rapidly when viewing objects successively (Uchikawa and Ikeda 1981, Dyer and Neumeyer 2005), and since a foraging herbivore is likely to encounter food in succession, this would aid in reducing a foraging herbivore's ability to discriminate between *A. pusilla* and *P. colorata* (Yager et al. 2016). Within a complex environment at the LH site where the availability of alternative plant food sources is relatively high, morphological similarity between neighbouring plants would advantage *A. pusilla* because any benefit to the herbivore from trying to distinguish between *A. pusilla* and *P. colorata* would be low. In contrast, at the HH site where the availability of alternative palatable species is lower, the benefit to a herbivore of distinguishing between the two species would be higher. A browsing herbivore is also more likely to encounter *A. pusilla* and *P. colorata* in multiple successions with little interaction with alternative species, leading to reinforced memory of any differences between the two species. With a higher pressure of herbivory it would be expected, therefore, that there would be a closer match in leaf shape between *A. pusilla* and its *P. colorata* neighbours. At the HH site *A. pusilla* individuals were morphologically similar to neighbouring *P. colorata* as well as to the population at large compared with the LH site (Fig. 3.6A-D). The leaves of both species were less variable (Table 3.3; Fig. 3.4A-B) and responded similarly across the elevational gradient at the HH site (Table 3.2; Fig. 3.5A, C-D). At the LH site, although *A. pusilla* individuals more closely resemble their neighbouring *P. colorata* than those growing further away, any differences between those *A. pusilla* individuals and the surrounding *P. colorata* at any given point are greater than compared to the HH site. This closeness in similarity of *A. pusilla* to the population of *P. colorata* across the shared growing range would make it even harder for a foraging herbivore to discriminate between them, especially with a high rate of successive encounters with the two species.

Batesian mimicry theory predicts that the degree to which a mimic resembles its model correlates with the proportion of mimics to models (Ruxton et al. 2004), whilst the relaxed selection hypothesis suggests that the level of mimetic accuracy correlates positively with the relative abundance of mimics to models and negatively with the availability of alternative prey (Sherratt 2002, Penney et al. 2012). With the higher abundance of mammalian herbivores, the unpalatable *P. colorata* became 43% more abundant and the palatable *A.*

pusilla 48% more abundant. This would be expected if introduced mammalian herbivores were unable to differentiate *A. pusilla* from the avoided *P. colorata*, whilst other palatable species favoured decreased in abundance. However, the proportion of *A. pusilla* to *P. colorata* (approx. 0.22 to 0.24) was similar for the LH and HH sites. While the leaves of *A. pusilla* and *P. colorata* at the HH site had a narrower range of variation in shape (Table 3.1; Fig. 3.4A-D), this did not increase the level of accuracy in leaf shape matching between the two species, with a similar proportion of individuals at each site misclassified (Table 3.3). Thus, although the resemblance between *A. pusilla* to *P. colorata* did not increase in the HH site, the decrease in *A. pusilla*'s leaf shape variation and closer response in shape variation magnitude over elevation may substitute for mimetic accuracy, allowing for the proportional increase in abundance of *A. pusilla*.

There appears to be two interactions working on variation in the leaf shape of *A. pusilla* and *P. colorata* brought about by the presence of introduced herbivores. Under low mammalian herbivory pressure, there would be less selection pressure on *A. pusilla* to match *P. colorata* than under high mammalian herbivory pressure, both because there are fewer predators as well as a greater abundance of alternative prey. The increased presence of alternative palatable species would also increase competition between the different plant species for resources as well as increase other known and unknown biotic and abiotic interactions in which the shape of leaves may play a role. Accordingly, leaf shape was most variable in both species at the LH site. Under high herbivory pressure, the removal of alternative palatable species would reduce competition pressures on *A. pusilla* and *P. colorata*. However, the removal of alternative palatable species would increase the scrutinisation of both *P. colorata* and *A. pusilla* by herbivores as they seek out palatable food sources. Under the relaxed selection hypothesis this would either lead to an increase in mimetic accuracy or, as seen in this study, drive the evolution of a more distinct signal of unpalatability in *P. colorata*, which in turn would be selected for in a mimicking *A. pusilla*. At the HH site most *A. pusilla* were found growing three times closer to individual *P. colorata* than within the LH site (Fig. 3.8), as would be expected if *A. pusilla* was under extra scrutiny from herbivores.

It is unknown what mechanisms may be involved in *A. pusilla*'s similar response in leaf shape variation to *P. colorata*, but it is probable that the two species use similar environmental cues. Equally, it is not known whether herbivory pressure was the primary force driving leaf shape convergence, or, instead, if this was primarily an adaptive response to abiotic stressors. However, in the presence of uncontrolled mammalian herbivores,

variation within the distinctive leaf shape of *P. colorata* and *A. pusilla* is reduced, as expected if stronger signals of unpalatability were being selected for. The removal of palatable species and avoidance of *P. colorata* has allowed for an increase in its abundance. Likewise, *A. pusilla* has increased in abundance proportionately as predicted if it was a Batesian mimic of *P. colorata*. It has been proposed that introduced species may substitute for extinct taxa by filling ecological niches previously occupied by native species (Jones et al. 1994, Griffiths et al. 2010), and it appears that recently introduced mammalian herbivores are now, at least, driving the selection for the similarity in leaf shape between *A. pusilla* and *P. colorata*. As there was little difference in the proportions of leaf laminae removed within areas of different proportions of *A. pusilla* to *P. colorata*, the level of invertebrate herbivory was similar between the two sites (Fig. 3.9). Although *A. pusilla* is a palatable species (Greenwood and Atkinson 1977, Nugent et al. 2001), these findings match field observations where it appears that it is often avoided with studies of rumen content of both red deer and goats finding *A. pusilla* is consumed significantly less than its relative availability (Mitchell et al. 1987, Nugent et al. 1997).

Chapter 4: Defensive Batesian mimicry deceives an introduced herbivore

4.1 ABSTRACT

Although introduced species are often associated with invasiveness, in some instances they may act as substitute species, functionally replacing a lost native species and the associated ecological interactions. In New Zealand, the large vertebrate herbivore niche was once dominated by the now extinct flightless ratites known as moa. Browsing pressure from moa has been attributed to the evolution of a number of distinctive plant defensive strategies. This herbivore guild was re-established by early European settlers with the introduction and naturalisation of mammalian herbivores. It has been suggested introduced deer may have functionally replaced moa, but to what degree is contentious. An example of purported moa-induced plant defence is Batesian mimicry; the vegetative shoots of the relatively undefended *Alseuosmia pusilla* are unusually visually similar to those of the chemically defended *Pseudowintera colorata*. Although this hypothesis is hard to test due to the extinction of moa, introduced deer avoid browsing *P. colorata* but will eat shoots of *A. pusilla*, and the similarity between the two might confuse deer. Unequivocal proof of mimicry in plants requires that: (i) both the mimic and a known model are attacked by the same herbivore, (ii) the herbivore is unable to distinguish between them, and (iii) this confusion increases the fitness of the mimic (Schaefer and Ruxton 2009, 2011). Using farmed red deer (*Cervus elaphus scoticus*), I conducted a feeding trial with *A. pusilla* and *P. colorata* sourced from a wild population to test the hypothesis that *A. pusilla* is a Batesian mimic of *P. colorata* by satisfying the three criteria above. Unusual deer feeding behaviour, not synonymous with wild deer behaviour was observed during the trials at night time, therefore feeding behaviour observations were restricted to the hours of daylight only. Deer not only ate less foliage overall from *P. colorata*, and were more likely to continue to eat an *A. pusilla* than a *P. colorata* individual, but deer became more cautious to eat another plant after having first eaten a *P. colorata* compared with having eaten an *A. pusilla* individual. As the proportion of *P. colorata* increased, the total browsing on both *P. colorata* and *A. pusilla* reduced, but more importantly, the amount of foliage remaining on individual *A. pusilla* increased. This effect was not seen with the other palatable trees. Finally, although the volatile organic compound emissions of the two species are very different, deer had an equal probability of making first feeding contact with either species across the trials. These results demonstrate that: (i) both *A. pusilla* and *P. colorata* are eaten by red deer, (ii) red deer are unable to distinguish between

them, and (iii) this confusion reduces the amount of browsing on *A. pusilla* individuals, and, can be inferred to increase the fitness of *A. pusilla* individuals. This is the first study that conclusively satisfies the criteria for Batesian mimicry in plants. Although deer do not share an evolutionary history with *A. pusilla* and *P. colorata*, this study also provides an interesting example of exaptive Batesian mimicry.

4.2 INTRODUCTION

The extinction of native species and the reduction of biodiversity can lead to the loss of ecological interactions in which those species were engaged (Tylianakis et al. 2008, Aizen et al. 2012). Likewise, the introduction of species to habitats beyond their native range can also cause changes to the ecosystems in which they have been introduced (Pyšek et al. 2012, Ricciardi et al. 2013). Although introduced species do not share an evolutionary history with the surrounding biota of their new habitat, they may naturalise and turn invasive, meaning they become numerically and ecologically prominent and potentially dominate native populations and communities (Crooks 2002).

Whilst invasive species will often have undesirable effects on native ecosystems, such as through alteration of food webs and physical environmental conditions (Ehrendfeld 2010), in many instances introduced species have had little or no effect on their new environment (Williamson and Fitter 1996, Pyšek et al. 2012, Strayer 2012). In other instances introduced species can have positive impacts on native biota by providing habitat and food for rare species (Schlaepfer et al. 2011), and may even act as substitutes for extinct native species, thereby re-establishing lost ecological interactions (Griffiths et al. 2010).

The large vertebrate herbivore niche in New Zealand was once dominated by the flightless ratites known as moa (Aves: Dinornithiformes) until their extinction by the mid-15th Century AD, not long after the arrival of humans (Perry et al. 2014). Moa consisted of nine species ranging between ca. 12 kg to 250 kg in weight (Bunce et al. 2009, Lee et al. 2010). They were found across all habitats (Worthy and Holdaway 2002), and being the predominant megaherbivores, would have strongly influenced the structure and composition of vegetation communities (Rogers et al. 2005, Lee et al. 2010, Wood et al. 2013). This missing large herbivore guild became re-established in New Zealand forests with the introduction and naturalisation of mammalian herbivores, such as the common brushtail possum (*Trichosurus vulpecula*), goats (*Capra hircus*), pigs (*Sus scrofa*), but especially deer (several *Cervus* spp.,

Dama dama, and *Odocoileus virginianus*), between 1850 and 1910 after the settlement of European settlers (King 1990, Fraser et al. 2000).

Several species of deer are now established throughout New Zealand, of which red deer (*Cervus elaphus scoticus* Lönnerberg) are the most widespread, and are of similar size to several extinct moa species (Worthy and Holdaway 2002, Forsyth et al. 2010). It has been argued that in some instances deer have functionally replaced moa (Caughley 1988, Batcheler 1989). However, to what degree is contentious due to hypothesised differences in ecological impacts (Atkinson and Greenwood 1989, Forsyth et al. 2010, Lee et al. 2010, Wood et al. 2013). For instance, the diet of deer does not fully overlap with the known diet of moa (Forsyth et al. 2010), and whilst deer will use olfactory cues when feeding (Elliott and Loudon 1987), moa likely had a poor sense of smell but good visual acuity (Ashwell and Scofield 2008).

Many New Zealand native plants have distinctive defensive strategies, which are hypothesised to have evolved as a response to browsing by extinct herbivorous birds, including moa (Atkinson and Greenwood 1989, Burns and Dawson 2009, Fadzly et al. 2009). This includes several putative cases of plant defensive mimicry (Dawson 1988, Brown and Lawton 1991), where an undefended species avoids predation by mimicking a trait or traits of a defended species (Ruxton et al. 2004). Arguably one of the most conspicuous examples is for *Alseuosmia pusilla* for which, in some of New Zealand's forest, the vegetative shoots cannot easily be visually distinguished from those of the unrelated *Pseudowintera colorata*, with which it is often sympatric (Yager et al. 2016). The leaves of *A. pusilla* contain only a few anti-herbivory compounds (Cambie and Parnell 1970); in contrast, those of *P. colorata* are rich in polygodial, a sesquiterpene dialdehyde that imparts a pungent taste and is a potent insect antifeedant (Asakawa et al. 1988). It has been suggested that the similarity in leaf morphology and leaf pigmentation is a response to browsing by moa, whereby *A. pusilla* evolved as a Batesian mimic to *P. colorata* (Greenwood and Atkinson 1977), though this hypothesis is difficult to test due to their extinction. Nevertheless, from rumen contents, faecal pellets and preference observations, introduced red deer avoid browsing *P. colorata* yet will eat shoots of *A. pusilla* (Nugent et al. 2001, Forsyth et al. 2003). It is possible, therefore, that the similarity between *A. pusilla* and *P. colorata* might confuse deer.

Batesian mimicry, a relatively common phenomenon in the animal kingdom (Ruxton et al. 2004), has yet to be demonstrated to function in plants (Schaefer and Ruxton 2009, 2011).

Successful plant mimicry requires that the mimic and model converge in the perceptual world of an approaching herbivore such that it cannot easily distinguish between the two (Schaefer and Ruxton 2009). How a herbivore is able to sense a plant can be estimated relatively accurately by studying the sensory systems of the animal. However, the cognitive ability of an individual herbivore is much more complex and is ultimately dependent on a level of subconscious trade-offs based on the benefits and costs of possible decisions (Abbott and Sherratt 2013). The degree to which a mimic resembles the model must, according to signal detection theory, be sufficiently great to reduce the motivation of the herbivore to attack the mimic (Sherratt 2002). At the critical level of resemblance, the motivation for a herbivore to risk attacking a model plant is influenced by the site specific mimic plant to model plant ratio. For example, when models are abundant relative to mimics, there is very little motivation for a herbivore to attack potential prey that even remotely resemble the model (Pfennig et al. 2001, Ruxton et al. 2004). Therefore in Batesian mimicry, the level of protection a mimic is afforded by its model is expected to be frequency-dependant.

The evolution of defensive mimicry in plants is theoretically possible, and may even be relatively common, depending on how reluctant a herbivore is to eat again after sampling a defended plant (Augner and Bernays 1998). Augner and Bernays (1998) found that mimicry could evolve if a herbivore shows any avoidance of individuals displaying a signal of unpalatability. In contrast, if a herbivore always samples a signalling defended plant, whether it is an honest signal or not, mimicry would never evolve. Unequivocal proof of mimicry in plants requires that: (i) both the mimic and a known model are attacked by the same herbivore, (ii) the herbivore is unable to distinguish between them, and (iii) this confusion increases the fitness of the mimic (Schaefer and Ruxton 2009, 2011). Although observed morphological similarities between two unrelated plant species have long been hypothesised as defensive mimicry (Brown and Lawton 1991), studies to date (Barlow and Wiens 1977, Shimshi 1979, Brown and Lawton 1991, Canyon and Hill 1997, Gianoli and Carrasco-Urra 2014, Lev-Yadun 2016), have only focused on documenting the shared similarities whilst overlooking how a shared herbivore responds to these similarities.

In order to test the hypothesis that *A. pusilla* is a Batesian mimic of *P. colorata* in the absence of moa, I used farmed red deer (*Cervus elaphus scoticus*) in a feeding trial type experiment in order to satisfy the criteria for proving plant mimicry as highlighted by Schaefer and Ruxton (2009, 2011). In doing so, I am also testing whether red deer are possibly maintaining an historical evolutionary selective pressure by acting as surrogate herbivores. By feeding *A.*

pusilla and *P. colorata* to deer at different proportions to one another, I hypothesised that: (i) *P. colorata* is avoided by deer more than *A. pusilla*, (ii) as the proportion of *P. colorata* increases in relation to *A. pusilla*, *A. pusilla* individuals will suffer less herbivory, and (iii) known palatable species that do not share a similar leaf shape and colour with *P. colorata* are readily eaten in the presence of *P. colorata*.

4.3 MATERIALS AND METHODS

4.3.1 Plant Material

All *A. pusilla* and *P. colorata* plants were sourced from a wild population. In total, 70 *A. pusilla* and 80 *P. colorata* plants were collected from the Mt Climie region of the Pakuratahi Forest, a montane forest reserve located in Upper Hutt, New Zealand (S41 7.30, E175 8.54). Plants between 30 cm and 50 cm in height were selected randomly as this was the most common height of mature *A. pusilla*, the smaller of the two species, from within 20 m of either side of the Mt Climie vehicle access road. Plants were then potted in planter bags and the soil topped up with commercial potting mix and acclimated outdoors under shade cloth shelters for 8 months at the Kelburn campus of Victoria University of Wellington.

The collection of plant material was approved with a High Impact Collection Permit from the Greater Wellington Regional Council.

4.3.2 Volatile Assay

To test how similar the two species may “smell” to deer, 10 leaves of each species were sent to the University of Otago for the analysis by Catherine Sansom (Plant and Food Research in association with the Department of Chemistry, University of Otago) for volatile organic compounds (VOCs) released by each species.

Plant material was sealed in a sample vial and the volatile compounds collected upon an adsorbent coating of the surface of a solid-phase microextraction (SPME, Supelco, Shanghai, China) fibre exposed to the air within the sample vial. Static headspace sampling via SPME is a rapid, cost effective and a sensitive technique for collecting VOCs (Koziel et al. 1999, Tholl et al. 2006). Analyses were then performed using a gas chromatograph-mass spectrophotometer (GC-MS Agilent Technologies, PA, USA).

4.3.3 SPME Absorption

The 65 µm Polydimethylsiloxane/divinylbenzene SPME fibre was conditioned at 250 °C in the GC injector port for 30 min before its first use and for 15 min at the start of each day.

A blank and a series of alkanes were analysed with the samples. A bulk sample of dried *P. colorata* leaf was analysed with each batch of dried leaf samples.

A fresh leaf, third or fourth from a branch apex, was ripped in quarters and placed in a 20 mL sample vial, sealed and placed in the autosampler tray. The fibre was immediately exposed to the sample at room temperature for 20 minutes.

4.3.4 GC-MS Procedure

Analyses were performed on an Agilent 7890A gas chromatograph with a CTC Analytics PAL system autosampler and an Agilent 5975C inert XL MSD with triple axis detector (under the control of Enhanced ChemStation software). The injector (260 °C) flow was splitless with a purge flow of 50 mL/min after 1 minutes. SPME desorption was for 1 minute onto a 30 m Agilent HP-5ms column with a 0.25 mm ID and 0.25 µm film, split between FID and MS detection using deactivated silica columns. The carrier gas used was hydrogen with a flow of 1.5 mL/min. The oven was heated from 40 °C to 250 °C at 5 °C/min. Detection was by mass spectrometry (MS). The MS transfer line was held at 260 °C, the MS source was held at 230 °C and the MS quad held at 150 °C, m/z 35-300.

Peaks were identified by comparing their retention time and mass spectra with those of standards, or by comparing their retention index and mass spectra with those in the Adams library.

4.3.5 Animal Subjects

Of the seven species of deer that are established in the wild in New Zealand, red deer (*Cervus elaphus scoticus* Lönnberg) are the most widespread (King 1990), and also the most commonly farmed. Feeding trials were carried out on farmed red deer located near Waikanae (S40 53.26, E175 3.29) north of Wellington, New Zealand between August 2013 and March 2014. Ethics approval for the use of live animals was obtained from the Victoria University of Wellington Animal Ethics Committee (approval 2012R8). Each deer had a unique numbered ear tag in one ear, which was used for identification. When ear tags were unreadable, markings on the body and the positions of the tags were used. Two moderately

sloped farm paddocks containing pasture grasses, predominantly perennial ryegrass, were used. One of these paddocks, the first to be tested, held 10 mature male deer; the second had 32 individuals comprising mature females and juvenile males and females after the females had fawned. Feeding data were collected from deer ranging between approximately 9 months and 4 years of age. Deer that were recently born and too young to receive identification ear tags were ignored from the study due to difficulty of identification; those deer seldom interacted with the plants used in the experiment. As the feeding trials were carried out on farmed deer, mature males were separated from females for breeding purposes. Prior to this experiment, all deer were unfamiliar with *A. pusilla* and *P. colorata* and their usual diet consisted of ryegrass supplemented with maize seeds. There were some pasture weeds present in the study site, these were *Verbascum thapsus* and *Cirsium arvense*.

4.3.6 Camera Equipment

Motion-triggered trail cameras were used to film interactions between deer and plants. A Little Acorn 6210MC (LTL Acorn Outdoors, Green Bay, Wisconsin, USA) and a Bushnell Trophy Cam (Bushnell Outdoor Products, Overland Park, Kansas City, USA), were set to take 60 s videos with a 3 s reset time. Video quality was set to 12 megapixels and the PIR (passive infrared) sensors on both units were set to “high” to ensure cameras were triggered when a deer was in the field of view. Cameras were able to record at night using infrared light. Cameras were attached to fence posts approximately 2 m from the plants therefore limiting the need to familiarise the animals to new structures. The farmer identified an area within each paddock where the deer would regularly congregate to maximise encounters with the plants.

4.3.7 Experimental Design

Because the farmed deer had only ever eaten ryegrass or maize feed, five sapling tree species known to be palatable to wild deer (Nugent et al. 1997, Forsyth et al. 2002) were presented to them to familiarise the animals to tree saplings as a new type of potential food source. The species were: *Griselinia littoralis*, *Pseudopanax crassifolius*, *Melicytus ramiflorus*, *Coprosma grandifolia*, and *Schefflera digitata*. A random mixture including members of all five known palatable species, were put out in a grouping of six in a 2 x 3 layout at the sites to be used for the feeding trials. Plants were changed every two days for one week irrespective of whether they had incurred browsing damage. During the second week the deer were introduced to six *P. colorata* plants only for 48 hours followed by six *A. pusilla* plants only

for 48 hours. The deer were introduced to *A. pusilla* and *P. colorata* separately in order to experience both species. As wild animals would have experience and be familiarised with both species, it was important to the palatability of each species was not novel to the farmed deer before the feeding trials began. Analysis of video footage indicates that all deer used in the feeding trial sampled at least one plant of each species during this time. The feeding trials began in the third week.

Each feeding trial consisted of a mixture of *A. pusilla* and *P. colorata* pegged out in their planter bags, 30-40 cm apart, for 48 hours. To test the mimicry hypothesis, *A. pusilla* and *P. colorata* plants were presented to the deer at the following proportions of *A. pusilla* to *P. colorata*: 0 (all *P. colorata*), 0.17, 0.25, 0.33, 0.5, 0.67, 0.75, 0.83, 1 (all *A. pusilla*). Six plants in a 2 x 3 layout were presented to the deer in the mature male paddock; four in a 2 x 2 layout were presented in the female and young male paddock. This difference was due to a need to maximise the number of feeding trials for statistical purposes for the limited number of plants I had available to use. The location of each plant species within the layout of presented plants varied randomly for each trial as decided by a coin toss. Two feeding trials were run consecutively each week during the experiment with a four month break when switching paddocks to allow for fawning. The location of the feeding trials within a paddock did not change during the duration of the experiment. After a plant was used in a trial, it was retired from further experiments irrespective of whether it was damaged or not. All deer within the paddock had access and were able to partake with each feeding trial (Fig. 4.1).

Fig. 4.1. A still capture from a trial video showing the 2 x 3 layout of the plants, the planter bags, and how they were pegged into the ground. To identify deer individuals, the yellow ear tags displayed a unique number, and other identifying features, such as body markings and/or the position of additional ear tags, could be used. Camera footage displayed date (month/day/year) and time (hours:minutes:seconds).

Video footage was reviewed, and for each feeding interaction between an individual deer and each plant, the duration of each interaction was recorded and the amount of foliage left on the plant was estimated. The time of day for each interaction was also noted. Feeding trials were discontinued once there were no longer enough plants that were undamaged by deer to make up a new trail.

In order to test whether deer in the presence of *P. colorata* would avoid other species that were palatable but which did not share a similar leaf shape, different ratios of *Hoheria sexstylosa* and *Griselinia littoralis* saplings (two known palatable tree species to red deer) were tested in the presence of *P. colorata* as control groups (for leaf images of palatable species, see Appendix C.1). The leaves of *H. sexstylosa* and *G. littoralis* are visually dissimilar in leaf shape, and all individuals of both species presented to deer in each feeding trial were of similar height. Each trial consisted of four plants in total and were made up of either *H. sexstylosa* or *G. littoralis* with *P. colorata* at the following proportions to *P. colorata*: 0.25 (three *P. colorata*), 0.5, 0.75, or 1 (no *P. colorata*). Individuals were pegged

out in a 2 x 2 layout and the total amount of damage to both species and *P. colorata* was recorded after being exposed to the deer for 10 hours of daylight. One feeding trial at each proportion was performed for *H. sexstylosa* and *G. littoralis*.

4.3.8 Statistical Analysis

Within the feeding trials, for un-paired data a Wilcoxon rank sum test was used whilst a Wilcoxon signed rank test was used for paired data. Generalised linear mixed models were used to test the effect of ratio and plant species on both the level of remaining foliage after deer browsing and deer reluctance to eat after sampling *P. colorata*, whilst a generalised linear models tested the effect of ratio on the remaining foliage of *A. pusilla* after deer browsing. To test for differences in the levels of compounds in the VOC profiles of *A. pusilla* and *P. colorata* leaves, I used a Wilcoxon rank sum test. All tests were carried out using R version 3.0.1 (R Core Team 2013).

4.4 RESULTS

4.4.1 VOC Emissions

There were significant differences in both the type and amount of VOCs emissions between the two species (Fig. 4.2). *A. pusilla* leaves only released green leaf volatiles therefore very few compounds were detected, whilst *P. colorata* had a wide range of volatiles including terpenes, monoterpenes and sesquiterpenes. A total of 65 compounds were detected within *P. colorata*, while only 13 compounds were detected in *A. pusilla*, 11 of which were shared between the two species. Of the 11 shared compounds, five compounds, including hexenal and 2-E-hexenal, were found between 1.6 to almost 61 times higher levels of concentration in *P. colorata*, and only one (compound 10) was 1.3 times higher in *A. pusilla* (Table 4.1).

Fig. 4.2. VOC emission profiles from leaves of *Alseuosmia pusilla* (dark bars) and *Pseudowintera colorata* (light bars). Mean $\pm$ S.E., n = 10 of each species. Asterisks indicates statistical significance between the level of compound in *A. pusilla* and *P. colorata*: *, p < 0.05; **, p < 0.01 (Wilcoxon rank sum test).

Table 4.1. VOC compounds found in *Alseuosmia pusilla* leaves that are either absent, or are found in concentrations that are less than (–), similar to (=), or greater than (+) in *Pseudowintera colorata* leaves. The level of difference in concentrations is a measure of “times greater” between each species mean concentrations for each compound. Asterisks indicate statistical significance on the Wilcoxon rank sum test *W* statistic.

Compound	Presence in <i>Pseudowintera colorata</i>	Level of concentration difference in compounds (x)	<i>W</i>	<i>P</i>
1	=	--	45	0.58
2	=	--	55	0.65
3	+	1.6	84	0.01*
6	+	3.3	82	0.02*
9	=	--	49	1
10	–	1.3	17	0.01*
11	=	--	53	0.85
hexenal	+	4.7	100	< 0.01**
14	absent	--	--	--
2-E-hexenal	+	60.9	100	< 0.01**
17	absent	--	--	--
19	+	4.3	90	< 0.01**
21	=	--	54	0.67

4.4.2 Feeding trials over 24 hours

Deer ate less foliage from *P. colorata* than *A. pusilla* over a 24 hour period; the median percentage of foliage eaten from individual plants was 100 % for *A. pusilla* and 40 % for *P. colorata* across all of the feeding trials (Wilcoxon rank sum $W = 828$, $p < 0.01$) (Fig. 4.3A). However, as the proportion of *P. colorata* individuals within a trial increased, there was no difference in the amount a deer individual browsed from individual plants of either species in the 24 h after the first deer had made contact with a plant; $Z = -0.66$, $p = 0.51$ (Table 4.2A).

Fig. 4.3. Box-and-whiskers plots of the amount of foliage consumed by deer from each trail over (A) 24 hours for *Alseuosmia pusilla* (n = 40) and *Pseudowintera colorata* (n = 66) plants. *A. pusilla* foliage was consumed at greater quantities than *P. colorata* (Wilcoxon rank sum $W = 828$, $p < 0.01$), and (B) 10 hours for *A. pusilla* (n = 40) and *P. colorata* (n = 66) plants. *A. pusilla* foliage was consumed at greater quantities than *P. colorata* (Wilcoxon rank sum $W = 733$, $p < 0.01$).

Table 4.2. Model formulae, coefficients, and standard errors, test statistics, and significance values from log transformed Poisson generalised linear mixed models for the amount of foliage browsed from *Alseuosmia pusilla* and *Pseudowintera colorata* as the proportion of *P. colorata* to *A. pusilla* increased over (A) 24 hour trials, and (B) 10 hour trials.

	Estimate	Standard error	Z-value	P-value
(A) Model: 24hourEaten ~ Plant + Ratio				
Intercept	4.54	0.02	224.66	< 0.01
Plant	-0.36	0.02	-19.59	< 0.01
Ratio	-0.02	0.03	-0.66	0.51
(B) Model: 10hourEaten ~ Plant + Ratio				
Intercept	1.37	1.42	0.97	0.33
Plant	0.42	0.65	0.65	0.52
Ratio	-0.03	0.01	-2.66	< 0.01
Individual deer were treated as random effects in these log transformed Poisson generalised linear mixed models.				

This initial result is inconsistent with the mimicry hypothesis, which posits that a mimic gains protection as the proportion of model increases. However, it was observed that some deer, especially the males, would return to the trial sites late at night to attack and often destroy the remaining plants of both species. Because this aggressive behaviour was done at night and therefore in the absence of visual cues as to the plant species identity, data analysis was restricted to a 10 hour period of daylight only after a deer's first contact with the plants, finishing at the latest 30 minutes before sunset. The next data analysis would use data collected from fresh plants from the next trial.

4.4.3 Feeding trials during daylight

For the observations conducted during daylight hours, deer preferentially avoided eating *P. colorata* compared with *A. pusilla*. The median percentage of foliage eaten from individual plants was 80 % for *A. pusilla* and 0 % for *P. colorata* across all of the feeding trials; demonstrating that for deer *P. colorata* was less palatable than *A. pusilla* (Wilcoxon rank sum $W = 733$, $p = 0.002$) (Fig 4.3B). As the proportion of *P. colorata* individuals within a trial increased, deer individuals browsed both species less overall; $Z = -2.66$, $p < 0.01$ (Table 4.2B). Overall, individual *A. pusilla* plants had less foliage eaten by deer as the proportion of *P. colorata* increased (Table 4.3; Fig 4.3).

Table 4.3. Model formulae, coefficients, and standard errors, test statistics, and significance values from a log transformed Poisson generalised linear model for the amount of foliage browsed from *Alseuosmia pusilla* as the proportion of *P. colorata* to *A. pusilla* increased over the 10 hour trials.

	Estimate	Standard error	Z-value	P-value
Model: 10hourAlsEaten ~ Ratio				
Intercept	1.71	0.94	1.82	0.08
Ratio	-0.03	0.01	-2.41	0.02

Fig. 4.4. The amount of foliage remaining on *Alseuosmia pusilla* after being exposed to deer browsing for 10 hours across different proportions of *A. pusilla* to *Pseudowintera colorata*. Each point represents one *A. pusilla* ($n = 40$), there may be multiple individuals at each point. A statistically significant regression equation was found, $F(1,38) = 7.33$, $p = 0.01$, $R^2 = 0.21$.

In contrast, the two known palatable tree species, *Hoheria sexstylosa* and *Griselinia littoralis*, both incurred a high level of browsing damage whilst *P. colorata* incurred very little damage, irrespective of how much *P. colorata* was present (Table 4.4). Across the different proportions of *P. colorata* to palatable tree species, deer ate similar proportions of foliage of both palatable tree species, *H. sexstylosa* ($Z = -0.70$, $p = 0.49$) and *G. littoralis* ($Z = 0.81$, $p = 0.42$) (Table 4.5).

Table 4.4. Species known to be palatable to red deer, *Hoheria sexstylosa* and *Griselinia littoralis*, were fed to deer at four different proportions to *P. colorata* (n = 1 trial at each proportion). The proportion of foliage removed on each plant for both the palatable species and *P. colorata* was recorded. Mean $\pm$ 95% C.I.

Species	Proportion	% "Palatable" foliage removed	% <i>P. colorata</i> foliage removed
<i>Hoheria sexstylosa</i>	0.25	90	15 $\pm$ 16.97
	0.50	80 $\pm$ 9.80	10 $\pm$ 20.00
	0.75	85 $\pm$ 9.80	5
	1	80 $\pm$ 12.00	-
<i>Griselinia littoralis</i>	0.25	85	5 $\pm$ 9.80
	0.50	98 $\pm$ 4.90	15 $\pm$ 0.00
	0.75	98 $\pm$ 3.27	5
	1	98 $\pm$ 4.90	-

Table 4.5. Model formulae, coefficients, and standard errors, test statistics, and significance values from a log transformed Poisson generalised linear model for the amount of foliage browsed from the palatable species (A) *Hoheria sexstylosa*, and (B) *Griselinia littoralis*, as the proportion of *P. colorata* to palatable species increased over the 10 hour trials.

	Estimate	Standard error	Z-value	P-value
(A) Model: HS ~ Ratio				
Intercept	4.48	0.11	41.29	< 0.01
Ratio	-0.02	0.03	-0.70	0.49
(B) Model: GL ~ Ratio				
Intercept	4.49	0.10	43.46	< 0.01
Ratio	0.03	0.03	0.81	0.42

Across the feeding trials, *A. pusilla* made up 49% of all plants and *P. colorata* the remaining 51% and deer were just as likely to eat either plant species on their first contact within a feeding trial (Wilcoxon test $V = 133.5$, $p = 0.184$). However, when a deer sampled a leaf they were significantly more likely to continue eating it if it was an *A. pusilla*, than if it was *P. colorata* (Wilcoxon test $V = 284$, $p = 0.023$). The first contact influenced a deer's willingness to attack another plant, with deer becoming reluctant to eat again after eating a *P. colorata*, taking longer to attempt to eat another plant compared with after having eaten an *A. pusilla*; $Z = 2.75$, $p < 0.01$ (Table 4.6; Fig. 4.5).

Table 4.6. Model formulae, coefficients, and standard errors, test statistics, and significance values from the log transformed Poisson generalised linear mixed model for the time taken to eat for an individual deer to eat another plant (either an *Alseuosmia pusilla* or *Pseudowintera colorata*), after its first contact (eating) in the trial was with either an *A. pusilla* or *P. colorata* individual during a 10 hour trial.

	Estimate	Standard error	Z-value	P-value
Model: Time ~ Plant + Ratio + Plant*Ratio				
(Intercept)	1.12	0.84	1.34	0.18
Plant	2.48	0.90	2.75	< 0.01
Ratio	1.42	1.08	1.31	0.19
Plant*Ratio	1.42	1.10	1.30	0.19
Individual deer were treated as random effects in this log transformed Poisson generalised linear mixed model.

Fig. 4.5. Box-and-whiskers plots for the time taken (in seconds) for an individual deer to eat another plant (either an *Alseuosmia pusilla* or *Pseudowintera colorata*), after its first contact (eating) in the trial was with either an *A. pusilla* or *P. colorata* individual ($n_{\text{PLANTS}} = 24$, $n_{\text{DEER}} = 9$). A deer took a statistically longer time to eat another plant again if its first contact was with a *P. colorata* individual than with an *A. pusilla* individual (see the generalised linear mixed model results in Table 4.6).

4.5 DISCUSSION

There were eight key findings from this study of deer browsing on *Alseuosmia pusilla* and *Pseudowintera colorata*. (i) *P. colorata* is better defended against deer browsing than *A. pusilla* as deer ate less foliage from *P. colorata* than from *A. pusilla* (Fig. 4.3B). (ii) When a deer had found an *A. pusilla* individual, it was more likely to continue to eat it than if it was a *P. colorata* individual. (iii) If the first plant a deer ate was a *P. colorata*, it became more cautious to eat any other plant, taking longer to eat again compared with after eating an *A. pusilla* (Table 4.6; Fig. 4.5). (iv) As the proportion of *P. colorata* relative to *A. pusilla* increased, total browsing on both species reduced (Table 4.2B). (v) *A. pusilla* individuals gained protection by growing alongside *P. colorata* as the amount of foliage remaining on *A. pusilla* individuals increased as the proportion of *P. colorata* to *A. pusilla* increased (Table 4.3; Fig. 4.4). (vi) Unlike with *A. pusilla*, deer were able to distinguish between *P. colorata*

and two known palatable tree species irrespective of how much *P. colorata* was present (Tables 4.4 and 4.5). (vii) *P. colorata* leaf volatile organic compound (VOC) emissions are more complex than those from *A. pusilla*, and of those emissions that are shared, those from *P. colorata* are generally at greater levels (Table 4.1; Fig. 4.2). (viii) Deer had an equal probability of making their first contact with either species. These results are consistent with the hypothesis that due to similar leaf morphology, *A. pusilla* is acting as a Batesian mimic to *P. colorata* for red deer.

Batesian mimicry requires that the model must be more unpalatable to the predator than the mimic (Ruxton et al. 2004). Across this experiment, deer avoided *P. colorata* often eating no foliage from individual plants; the median amount of foliage eaten was 0%, while for three-quarters of all *P. colorata*, deer removed 50% or less of the available foliage. In contrast the median level of foliage eaten from *A. pusilla* individuals was 80% (Fig. 4.3B). After having begun to eat an *A. pusilla* individual, deer were also more likely to continue to browse on that individual compared to when having begun to eat a *P. colorata* individual. This demonstrates that *A. pusilla* will be readily eaten by deer, and therefore more palatable than *P. colorata*. The avoidance of *P. colorata* is consistent with data from deer field exclusion plots where *P. colorata* is found to be more abundant outside plots (Allen et al. 1984, Husheer et al. 2003), its absence from ungulate rumen analyses (Nugent et al. 1997), and dominance as an understory species where high populations of deer exist (Allen et al. 1984). Conversely, preference data based on the relative frequency of *A. pusilla* ingested by deer compared with what is available in the study area show *A. pusilla* as being “moderately palatable” (Nugent et al. 2001, Forsyth et al. 2003). It should be expected in field preference studies that if *A. pusilla* was a Batesian mimic of *P. colorata*, that *A. pusilla* would be found to be either “avoided”, or “moderately palatable” rather than “highly palatable” as deer would naturally avoid it, especially in the presence of *P. colorata*. Therefore, it was important to conclusively identify whether deer would readily eat *A. pusilla*.

After having browsed on *P. colorata*, deer became more cautious to browse again compared with after having browsed an *A. pusilla* individual, illustrated by a greater latency to eat again (Table 4.6, Fig. 4.5). This reluctance to eat again after sampling a defended model is one of the conditions required for Batesian mimicry to evolve in plants as modelled by Augner and Magnus (1998). However, Batesian mimicry is frequency-dependent. That is, as the risk of accidentally eating a model decreases when the proportion of mimics to models increases, the effectiveness of the shared signal of unpalatability in which Batesian mimicry is dependent

on also decreases (Brower and Brower 1962, Pfennig et al. 2001). The proportion of *P. colorata* relative to *A. pusilla*, reduced the level of browsing by individual deer on both *A. pusilla* and *P. colorata* individuals (Table 4.2B). More importantly, deer tended to increasingly avoid *A. pusilla* individuals as the proportion of *P. colorata* to *A. pusilla* increased, demonstrated by the reduction in browsing on *A. pusilla* individuals (Table 4.3, Fig. 4.4). Deer consumed most of the foliage available from the two known palatable tree species, *Hoheria sexstylosa* and *Griselinia littoralis*, even as the proportion of *P. colorata* increased (Tables 4.4 and 4.5). Therefore, deer were able to browse on either of the known palatable species whilst avoiding *P. colorata*, and only *A. pusilla* was dependent on the frequency of *P. colorata* as predicted by Batesian mimicry.

Although *A. pusilla* and *P. colorata* are visually similar in leaf shape and colouration (Yager et al. 2016), deer will often use olfactory cues when feeding (Elliott and Loudon 1987). There were significant differences in VOC emissions between *A. pusilla* and *P. colorata* indicative that the two species may smell very different from one another. *P. colorata* released a much wider range of volatiles than *A. pusilla* and generally at much higher levels including a range of terpenes (Fig. 4.2). The sesquiterpene Polygodial is responsible for the pungent pepper taste and odour in *P. colorata* (Szallasi et al. 1996), however the taste and odour of other terpenes such as Sabinene and α -Pinene also present in *P. colorata* have been found to strongly repel deer from feeding (Vourc'h et al. 2002). However, deer appear to be unable to use these olfactory differences to discriminate between the two species. Throughout this experiment, deer were just as likely to make first contact with a *P. colorata* individual as they were with an *A. pusilla* individual. It is possible then that the volatiles from *P. colorata* mask or hide those from *A. pusilla*, especially as the proportion of *P. colorata* increases. Plant volatiles, including terpenes, can mask other “attractive” volatiles from invertebrate herbivores, even when they are released from another plant (Yamasaki et al. 1997, Schröder and Hilker 2008). However, if the VOC's from *P. colorata* act as a mask, it is restricted to masking those from *A. pusilla*, as both *H. sexstylosa* and *G. littoralis* were readily eaten irrespective of the proportion of available *P. colorata*.

The frequency-dependent prediction did not hold when the study included data from non-daylight hours. During this time, both *A. pusilla* and *P. colorata* were extensively browsed irrespective of the proportion of *P. colorata* to *A. pusilla* (Table 4.2A; Fig. 4.3). Male deer were observed to be more interested in *P. colorata* than females, spending more time smelling, tasting and browsing the same individual plant. This may be due to female deer

having a stronger avoidance of terpenes (terpenes are present in *P. colorata* (Corbett and Grant 1958)) than males (Elliott and Loudon 1987). Males also demonstrated unusual behavioural patterns at night, becoming aggressive towards the plants, pulling them from their plastic planter bags, and even trying to eat the bags. It is possible that at night when visual cues are less distinguishable, that deer are less visually discriminatory. However it is probably unlikely that in the wild in the presence of a variety of sympatric palatable species, and where field studies show *P. colorata* is strongly avoided (Nugent et al. 2001, Forsyth et al. 2003), that the observed behaviour of the males in this study would continue to occur.

This study of shared leaf shape between *A. pusilla* and *P. colorata* demonstrates that: (i) both *A. pusilla* and *P. colorata* are eaten by red deer, (ii) red deer are unable to distinguish between them, and as herbivory can have a negative effect on plant fitness (Marquis 1984, Crawley 1985, Hawkes and Sullivan 2001), (iii) this confusion reduces the amount of browsing on *A. pusilla* individuals, and, can be inferred to increase the fitness of *A. pusilla* individuals. This evidence therefore satisfies the requirements for defensive Batesian mimicry within plants as laid out by Schafer and Ruxton (2009, 2011).

As deer do not share an evolutionary history with either *A. pusilla* or *P. colorata*, this study is also an example of exaptive Batesian mimicry. The functionality of the similar leaf morphology and colouration between the two species has been co-opted as a defensive strategy that deters introduced deer. The visual similarity of *A. pusilla* to *P. colorata* has been long hypothesised to have evolved under selective pressure from browsing moa (Greenwood and Atkinson 1977), a dominant grouping of several now extinct species of avian mega-herbivores (Worthy and Holdaway 2002, Bunce et al. 2009). Moa likely had a poor sense of smell but good vision (Ashwell and Scofield 2008), and colour and form therefore may have been important traits used by moa in recognising palatable food (Atkinson and Greenwood 1989). As far as I am aware, to date no studies have found either *P. colorata* or *A. pusilla* remains in any moa coprolite (dried droppings) or gizzard remains. Therefore it is quite possible that *A. pusilla* did indeed evolve as a Batesian mimic of *P. colorata* to dupe moa, and possibly other extinct herbivorous bird species.

Several distinctive plant growth characteristics across a taxonomically diverse range of New Zealand flora, including divarication, heteroblasty as well as mimicry and others, have been attributed to moa-plant co-evolution (Greenwood and Atkinson 1977, Atkinson and Greenwood 1989, Batcheler 1989). However, to what degree deer have functionally replaced

moa within the whole ecosystem is a contentious topic (Atkinson and Greenwood 1989, Forsyth et al. 2010, Lee et al. 2010, Wood et al. 2013). The diet of moa is very similar to that of deer. Approximately 73% of plant taxa recorded to date in the diet of moa is also found in the diet of deer (Forsyth et al. 2010, Lee et al. 2010). While moa probably had a similar dietary intake rate of dry matter as red deer individually, in any given area, red deer are able to reach population densities 10 times or greater than moa (Forsyth et al. 2010). The feeding strategy of deer and moa also differs; deer are able to manipulate food using soft lips and tongues and moa had hard beaks requiring plucking or snipping of their food (Bond et al. 2004). Neither *A. pusilla* nor *P. colorata* have any structural deterrents that would deter one feeding strategy over the other. Deer tend to avoid plants with divaricate architecture (Pollock et al. 2007), and the content of moa gizzards and coprolites support the moa-plant co-evolution hypothesis for many distinctive plant growth forms (Wood et al. 2008). Therefore, although deer potentially exert a greater herbivory pressure than moa due to greater population densities, deer may still act as substitute species in as much as through maintaining uniquely evolved anti-herbivory characteristics, including instances of Batesian mimicry.

It is possible that the visual similarity of *A. pusilla* to *P. colorata* may equally successfully deter browsing by other introduced generalist mammalian herbivores. Whilst this study focused specifically on red deer, after European settlement, possums, goats, pigs, hares and several other species of deer were successfully established in New Zealand forests (King 1990, Fraser et al. 2000). The successful establishment of many of these species is partially due to a lack of predators, but also as they are able to consume a wide variety of plant species (Parkes 1993, Forsyth et al. 2010), much like moa (Forsyth et al. 2010, Wood et al. 2012). If this is the case, visual deception may be an overlooked and broadly effective plant defensive strategy against generalised herbivores, especially for those that have shared no evolutionary history. Visual cues to roughly discriminate between plants and patches can be used by many large browsing and grazing herbivores (Searle and Shipley 2008) and insects (Reeves 2011), but the level of accuracy may probably varies between species. For visual deception in plants to be common, it would be expected that shape discrimination would be relatively basic and the similar across taxonomic groups of generalist herbivores. However, a greater understanding of how generalist herbivores are able to utilise shape discrimination is required.

Chapter 5: The use of leaf traits to discriminate between a purported mimic and its model

5.1 ABSTRACT

Leaf shape, size and colour are used by herbivores to identify sources of palatable foliage for food. However, how herbivores use these plant traits as signals and cues, when they use them, and the factors involved in how they learn about them have not been studied. Signals are often complex, made up of multiple traits. They require feedback to the signal receiver in order to learn associational benefits and/or costs. They are often presented amongst a distraction of other uninformative “noise”, and alongside other similar signals that can create a level of uncertainty in the discrimination task. Using the images of real leaves from two plant species that are similar in shape and colour, the chemically defended *P. colorata* and its purported mimic, *A. pusilla*, I engage human subjects as surrogate herbivores in a computer based discrimination task to forage for *A. pusilla* leaves whilst avoiding those from *P. colorata*. I test how leaf shape and leaf colour are used independently and together as discriminable traits. I also test how factors such as feedback speed, frequency of mimic to model, and a visually “noisy” background affect their discrimination abilities. Humans, as surrogate herbivores, found leaf size a distracting characteristic, foraging more effectively when *A. pusilla* and *P. colorata* individuals were most similar in 94% of their shared shape variation. The trait, leaf colour, whilst unreliable by itself, acted to potentiate the trait, leaf shape, as a signal or cue. Fast feedback on species palatability improves accuracy on discriminating *A. pusilla*, but neither fast nor slow feedback improved discriminability on *P. colorata*. Finally, *A. pusilla* leaves were harder to discriminate when presented on a “disruptive” background. This study demonstrates that although plants may be sessile organisms, the visual complexity and number of factors that interact with signalling between plants and animals is multifaceted. Whilst this study provides interesting insight into how leaf traits can be used to discriminate between visually similar species, it also highlights the need for further studies in the field of plant-herbivore visual communication.

5.2 INTRODUCTION

Herbivores are dependent on plants for food, removing more than 20% of annual net primary productivity across biomes, habitats and natural and managed systems (Agrawal 2011). This pressure on plants has fuelled an ongoing co-evolution between plants and animals; plants

have evolved a range of defensive strategies to reduce the impact of herbivores, whilst herbivores evolve counter measures to overcome these increased defences (Ehrlich and Raven 1964, Karban and Agrawal 2002, Agrawal 2011).

Leaf shape and size have long been hypothesised to play a role in deterring herbivores (Brown and Lawton 1991). Although leaf morphology assists with regulation and mitigation of the effects of environmental stressors such as shoot temperatures (Hegazy and El Amry 1998, Roth-Nebelsick 2001), light interception (Horn 1971, Tsukaya 2005), avoidance of wind damage (Anten et al. 2010), salt exclusion (Sinclair and Hoffmann 2003), drought avoidance, or nutrient deficiencies (Cunningham et al. 1999, Leigh et al. 2011), the diversity of leaf shape and size in plants suggests that it is not exclusively functionally dependent on any one ecological strategy (Nicotra et al. 2011). Leaf shape has, however, been demonstrated to influence the consumption, performance, and ovipositioning choices of insect herbivores (Rausher 1978, Mackay and Jones 1989, Rivero-Lynch et al. 1996, Campitelli et al. 2008a). Leaf colouration may also deter herbivores by acting as a visual cue of a plant's palatability (Green et al. 2015). Autumnal leaf colouration may signal increased defences and/or low nutrient quality to egg-laying insects (Hamilton and Brown 2001, Archetti and Brown 2004, Archetti 2009b), whilst red leaf margins can signal defensive investment and deter insect herbivores (Cooney et al. 2012, Hughes and Lev-Yadun 2015). Leaf shape, too, can provide a visual cue of a plant's palatability (Dell'aglio et al. 2016); therefore, leaf shape and colour may act as distinctive signals for herbivore recognition of palatability.

The definition of a signal is a trait or traits that convey information to other organisms and are maintained by natural selection; cues, on the other hand, are defined as informative traits that evolved for reasons other than communication (Otte 1974). If herbivores learn to avoid traits associated with unpalatability and these traits were not initially associated with communication, they may be co-opted to become new communication signals, a process known as ritualization (Tinbergen 1952, Huxley 1966, Lorenz 1966). Experiments show that some animals may only use one or a few signals when discriminating among visual trait characteristics (Troje et al. 1999). This may be due to an innate avoidance of a particular colour or pattern due to perceptual or cognitive biases of the predator (Rowe 1999). Alternatively, an animal may favour using one trait over others as a speed-accuracy trade-off (Hebets and Papaj 2005), or one trait may "overshadow" another trait if it is more salient (Mackintosh 1976). Therefore, signal displays may evolve to be relatively simple.

However, signals are often complex and are made up of multiple traits that require different cognitive attention (also referred to as multidimensional) (Hebets and Papaj 2005, Balogh et al. 2010, Kikuchi et al. 2016). For example, the wings of defended butterflies display a combination of colours and patterns (Bates 1862), as do the bodies of many venomous snakes (Greene and McDiarmid 1981, Niskanen and Mappes 2005, Valkonen et al. 2011). The use of multidimensional, rather than single dimensional, signals by animals probably conveys different sets of information that could, for example, enhance a predator's ability to learn and/or remember, or to increase their detection accuracy (Hebets and Papaj 2005). Individual traits within the multidimensional signal can function independently within the cognitive processes of an animal predator. They may be: (i) of equal salience, (ii) emergent; combining to create a new signal, (iii) contextual, wherein one trait provides context to interpret a second trait, (iv) alerting; drawing attention to a second signal, or (v) amplifying; increasing the probability of detection of a second trait and thereby increasing overall conspicuousness (Hebets and Papaj 2005).

Before an animal can cognitively process a signal, they must distinguish the signal from other competing sensory information, referred to as noise. A signal that is conspicuous can stand out from the noise, making it faster to learn (Gittleman and Harvey 1980, Roper and Redston 1987, Lindström et al. 2001), and therefore easier to discriminate from less conspicuous signals (Roper and Redston 1987, Lindström et al. 1999, 2001). However, not all signals are conspicuous. Prey can be non-conspicuous or cryptic and avoid detection by blending in with their surroundings through varying forms of camouflage. Indeed, conspicuousness can draw unwanted attention, especially from naïve predators, and therefore undefended or poorly defended species would benefit by being cryptic (Ruxton et al. 2004). Defended prey can also benefit from avoiding extra attention by being non-conspicuous or even cryptic as long as their signal is distinctive; that is, different enough from surrounding species to be recognisable if found by a predator (Sherratt and Beatty 2003, Merilaita and Ruxton 2007).

Once nutritional associations have been learned, herbivores often use pre-ingestive information, e.g. visual, chemical or tactile cues, to recognize specific plants (Launchbaugh and Provenza 1993, Augner and Bernays 1998, Schoonhoven et al. 2005), and can generalise their aversions across familiar cues in defended plants (Launchbaugh et al. 1993, Villalba and Provenza 2000). Other herbivores may use post-ingestive information, e.g. satiation, to regulate their food intake (Provenza 1995). Whilst these two feedback mechanisms are not mutually exclusive, Augner and Bernays (1998) point out that in some cases, such as when a

plant is highly toxic or there are long handling times, it may be more beneficial for a herbivore to use pre-ingestive cues, known as defensive signals, rather than post-ingestive feedback as it reduces risk and shortens decision times.

In some instances signals evolve to be dishonest. One such occasion is when an undefended species copies the signal associated with defence of another species, a phenomenon known as Batesian mimicry. In order to dupe a signal receiver, the mimicked signal requires a certain degree of similarity to that of its model. However, whilst it is assumed that mimics always experience selection to more closely resemble their models (Ruxton et al. 2004), imperfect mimicry is relatively common (Kikuchi and Pfennig 2013). Successful mimicry requires that the mimic and model converge in the perceptual world of a predator such that it cannot easily distinguish between the two (Schaefer and Ruxton 2009), thus creating a level of uncertainty. The frequency-dependency hypothesis suggests that selection for the accuracy of mimicry is relaxed as the proportion of models increases, and likewise, if mimics are too common the protection through predator deception breaks down as predators learn to ignore the warning signal (Pfennig et al. 2001, Ruxton et al. 2004). Variations in the accuracy of mimicry may also be due to fitness trade-offs associated with maintaining the signal, such as reduced ability to compete for resources (Pfennig and Kikuchi 2012).

Uncertainty typically arises from perceived similarity in the appearance of stimuli (e.g. Sherratt 2001, Leonard et al. 2011), such as in cases of non-conspicuous prey and Batesian mimicry complexes. The use of signals by animals under conditions of uncertainty is commonly studied using signal detection theory (SDT). SDT provides a framework to quantify a signal receiver's ability to discriminate between profitable (target) and unprofitable (distractor) stimuli that maximises net payoff and incorporates both the benefits of correct decisions (hits and correct rejections) as well as the costs of false positives (false alarms) and false negatives (misses) (Kikuchi et al. 2015).

McGuire et al. (2006) used computer-generated "prey", on human subjects to demonstrate the applicability of SDT in relation to Batesian mimicry in a singular dimension. Prey consisted of rectangular shaped "models" and "mimics" that varied in the number of different coloured pixels. Expanding on that work, Kikuchi et al. (2015) also used human subjects and the same computer generated prey to demonstrate that SDT could be used to test multidimensional signals, and highlighted the importance for further research in the application of SDT to multidimensional problems in ecology. The use of humans as surrogate predators is common

in the study of crypsis, warning signals and mimicry (e.g. Sherratt and Beatty 2003, Beatty et al. 2005, McGuire et al. 2006, Kikuchi et al. 2015), and there is good qualitative congruity found between the performance of humans and other animals such as birds when performing simple discrimination tasks (e.g. Dittrich et al. 1993, Beatty et al. 2005). The use of artificial prey, e.g. computer generated, which allows for easy control of different aspects of tested traits, has also been commonly used to date to understand how they are used as signals (e.g. Ihalainen et al. 2007, 2012, Kazemi et al. 2014, 2015, Kikuchi et al. 2015, 2016, Sherratt et al. 2015). Therefore, the number of replicated predatory decisions by using human surrogate predators and the control over trait aspects of prey that computers allow for is extremely difficult to achieve when using other animal species and physical prey items.

The leaves of two New Zealand native plant species, *Alseuosmia pusilla* (Colenso) A. Cunningham (Alseuosmiaceae) and *Pseudowintera colorata* (Raoul) Dandy (Winteraceae), share a remarkable degree of similarity in both leaf colour and leaf shape (Yager et al. 2016), and it is hypothesised that at locations where the two plant species co-occur, *A. pusilla* may escape predation by effectively functioning as a Batesian mimic of *P. colorata* (Dawson 1988, Atkinson and Greenwood 1989). This similarity between the two species make them ideal to use SDT to identify how traits that contain natural variation, rather than computer generated prey, are used by surrogate herbivores as cues or signals, and therefore, how the use of multidimensional traits are directly applicable to a real ecological situation. However, the putative herbivores that are thought to have selected for this similarity, several species of large herbivorous flightless birds known as moa (Dawson 1988, Atkinson and Greenwood 1989), are extinct and cannot be used to test how they may use leaf shape characteristics to discriminate between the two species.

Therefore, in this study human subjects representing surrogate herbivores were asked to discriminate between images of real leaves, referred to as “models” or “mimics”, which varied in either only shape, only natural colour, or in shape and natural colour. As humans and birds have been found to perform similarly on discrimination tasks and have been used by other studies as surrogate herbivores, I use them in this study to test how the abilities of subjects to discriminate between *P. colorata* and *A. pusilla* are affected by: (i) different leaf traits and how they interact; (ii) the variation in the overall similarity of leaf shape between *P. colorata* and *A. pusilla*; (iii) the frequency of models to mimics; (iv) the speed of feedback on selections; and (v) the level of conspicuousness of the leaves.

5.3 MATERIALS AND METHODS

5.3.1 Plant Material

Plant material collection and sampling technique was the same as described in section 2.3.1 of Chapter 2.

5.3.2 Leaf Shape Capturing and Analysis

Cartesian coordinates of 70 landmarks evenly distributed around the margin of each leaf image were transformed into partial Procrustes coordinates using the software package PAST 2.17c (Hammer et al. 2001) to minimise differences in leaf orientation of the scanned images without scaling for leaf area. Leaf shape was quantified by LeafAnalyser 2.3.0 (Weight et al. 2008) using the partial Procrustes coordinates. A principal component analysis (PCA) was then run using the 70 partial Procrustes coordinates, reducing the number for each species into four principle components (PC) that explained the majority of shape variation for each leaf. In order to create leaf groups that were similar in leaf shape (*Similar*) and dissimilar in leaf shape (*Dissimilar*), the PC1 and PC2 scores of each leaf were used to calculate the mean PC1 and PC2 values and a 1 standard deviation (S.D.) ellipsoid from the mean for both species (see section on *Prey* below for further details). Adobe Photoshop 5.0 (San José, California, USA) was used for post selection adjustments of leaves for presentation to survey participants; painting leaves to create leaf silhouettes and creating square portions of leaf laminae.

5.3.3 Survey Setup

Fourteen surveys were set up (Fig. 5.1) on an online web based survey website hosted by Qualtrics (Qualtrics, Provo, Utah, USA) with functional modifications made using JavaScript. The on-line format was used in order to access a large pool of participants. Each survey comprised 9 screens. Each screen had 24 images of leaves proportionately sized and scaled to one another, the relative sizes of the leaves were the same irrespective of the participants' computer screen size, and leaves were evenly distributed over a white background in a 4 row by 6 column arrangement (hereafter known as a "screen") (Fig. 5.2). Each screen was a composite of the images of *A. pusilla* and *P. colorata* leaves arranged in a ratio of 1:3 (6 x *A. pusilla* and 18 x *P. colorata*), 1:1 (12 x *A. pusilla* and 12 x *P. colorata*), or 3:1 (18 x *A. pusilla* and 6 x *P. colorata*). All leaf images were oriented with their petioles pointing downwards, and the location of each leaf image, although pre-set within the

arrangement for each screen (see *Prey* section below for more information), was randomly assigned when designing the survey (Fig. 5.2). Leaf image locations for each screen were assigned a number 1 – 24 and each image was randomly assigned using the random number function in Microsoft Excel (2013).

Fig. 5.1. Assignment of the 14 surveys and the variables that were tested. There were two survey types issued, “Plain Background Survey” and “Disruptive Background Survey”. Survey assignment was randomly allocated below the “Background type” level by Qualtrics. The term “dimensional” describes the number of traits that require different cognitive attention.

Fig. 5.2. An example of the same screen presented to subjects across the three trait levels being tested within the *Similar* survey with no “disruptive” background image; (A) leaf colour only, (B) leaf shape only, and (C) leaf colour and shape. Location of individual leaves within each screen were the same between each trait being tested, but the order in which the screens were presented to subjects was random. The type of survey a subject was given was randomly allocated by the survey programme, Qualtrics, including whether feedback on subjects choices was instant or delayed.

Surveys were randomly assigned to participants by the Qualtrics survey programme (hereafter referred to as “subjects”); they consisted of images of model and mimic leaf groups that had been categorised as either *Similar* or as *Dissimilar* in leaf shape (Fig. 5.1). To understand which leaf traits were used by subjects to distinguish between mimics and models (Plain Background Survey; Fig. 5.1), subjects received 9 screens of images containing either: (i) uniformly-sized coloured squares taken from portions of the leaf laminae (to test for the exclusive use of leaf colour; Fig. 5.2A); (ii) silhouettes of the leaves digitally painted a uniform green (to test for the exclusive use of leaf shape; Fig. 5.2B); or (iii) unaltered images of the leaves to test how leaf colour and leaf shape combined were used (Fig. 5.2C). Within the *Similar* and *Dissimilar* leaf groupings, subjects were given surveys that tested a single leaf trait (leaf shape or leaf colour) or ones that tested for the effects of both traits in combination (leaf shape and colour) (Fig. 5.1).

A scoring system in which subjects received points for selecting a mimic and lost points for selecting a model provided feedback (see *Standard Conditions* section below for more information). Feedback on a subjects’ selection via the scoring system was either provided immediately in a pop up box after each selection of a leaf was entered, or else feedback was delayed until an entire screen had been submitted. Separating how feedback was provided enabled me to test how delayed or instant feedback affected a subject’s accuracy in identifying mimics and models.

5.3.4 *Predators*

Volunteer human subjects were sourced from first- and second-year undergraduate university Biology courses during 2014 and 2016 at Victoria University of Wellington and Auckland University. Ethics approval for the use of human subjects was obtained from the Victoria University of Wellington Human Ethics Committee (approval 19370). The potential subjects had been told how long the survey would likely take to complete, and that the intention of the survey was for PhD research into how leaf colour and leaf shape influences leaf identification. Five invitations were sent out for survey applicants, both via direct email to students and by Blackboard (course related website pages), repeating the intentions of the survey and containing the web address URL to the survey. Subjects were then assigned one of the 14 surveys (Fig. 5.1). SDT is primarily concerned with identifying the optimal behaviour of predators who are already familiar with the model and mimic. Therefore, before beginning the survey, subjects were first shown one screen containing a panel that had 15

examples of leaves from the mimics, and then another with 15 examples of models; these leaves were randomly chosen from all available leaf images and included four images that were used within the survey. The presented images making up the panels were either from the *Similar* or *Dissimilar* leaf groupings and were representative of the appearances of mimics and models the subject was about to experience (Fig. 5.3). Subjects were instructed to spend as long as they wanted to examine this display in order to learn to identify the two species in order to make sure they were familiar with the mimic and model.

Fig. 5.3. The screen used for the leaf colour and shape and leaf colour only *Similar* grouped surveys shown to subjects before they started identifying mimics. Subjects who were to be presented the survey testing the trait leaf shape only were presented a similar screen but leaves were of a uniform colour as depicted in Fig. 5.2B. Subjects were allowed to spend as long as they wanted to “learn” differences between the two species. Images of leaves that depicted the traits leaf colour and shape were replaced with images of leaves with either the trait leaf shape and colour or leaf colour only for participants given the corresponding versions of the survey.

5.3.5 Prey

Individual leaves were categorised as *Similar* or *Dissimilar* based on leaf shape characteristics only (not colour) from their PC1 and PC2 scores. PC1 and PC2 accounted for 93.7% of the total leaf shape variation, with most (89.6%) attributable to variation in leaf size and to size-associated shape differences represented by PC1. PC2 accounted for 4.1% of the

total variance in shape and represented the morphological progression from a left-biased asymmetric elliptic lamina through to an asymmetric right-biased cuneate lamina. The *Similar* group comprised model and mimic leaves that lay within 1 S.D. of their PC1 and PC2 mean leaf shapes. Each screen therefore contained leaves with minimal difference in PC1 and PC2 scores. The *Dissimilar* group was made up of leaves from outside 1 S.D. of the mean leaf shapes, but for each screen, the presented leaves of mimics were matched such that there was maximum difference in PC1 and PC2 scores from the presented leaves of models (for further information, see Appendix D). However between the screens, PC1 and PC2 scores varied between mimics and models; for example, the size, and the shape parameters associated with size (PC1) of the mimic leaves within each screen varied in relation to those of the models within the *Dissimilar* group.

Because lamina colour is similar between the two species and leaf shape was more variable (Yager et al. 2016), for these experiments, when testing leaf colour I allowed for the natural differences in colour to vary. Therefore, similarity of leaves (*Similar* or *Dissimilar* leaf groupings) was based on leaf shape only, and I was interested in how the natural variation in leaf colour between mimics and models would affect subjects' discrimination abilities between the two groupings. When testing if subjects were able to discriminate between models and mimics based on leaf colour only, 105 x 105 pixel sized portions of the original *Similar* or *Dissimilar* leaf were selected from the centre of each leaf image lamina (Fig. 5.2).

Images were scaled in size, but within the set configuration of a 4 x 6 arrangement, accordingly by the subject's computer. The locations of models and mimics were pre-set for each screen, but the order of the screens presented to subjects was randomly assigned by the survey programme (Fig. 5.2). Each prey item attacked (if any) and their overall score was recorded per screen for each respondent.

5.3.6 Standard Conditions

For the *Similar* and *Dissimilar* surveys, subjects were presented images of leaves of the following traits, either: leaf colour only, leaf shape only, or leaf colour and shape (Fig. 5.2) and feedback on their selections as they progressed through the survey was either instant or delayed (Fig. 5.1). The subjects were shown a selection of models and mimics to learn for themselves any identifying differences and, when they were ready, asked to select all "Species A" leaves (the mimic - *A. pusilla*) on each successive screen. For the correct selection of a mimic, subjects were awarded 1 point, and for every incorrect selection – a

“Species B” leaf (the model - *P. colorata*) – they lost 1 point. Subjects were provided with a prize incentive and were initially told those with the top scores would receive a prize of a \$20 music voucher as motivation to maximise their score. Subjects were then asked to “forage” in a way to maximize their score. They were then invited to start the experiment. After a subject had completed the survey it was revealed to them that every subject had an equal chance to win one of the ten vouchers as their participation was valued.

There was no set limit to the amount of foraging time for a subject. A subject could decide whether to attack any prey item (by moving the mouse over the item and clicking), or move on to the next screen (by pressing the “Next” button). Subjects were unable to go back to a previous screen. For those subjects given instant feedback, attacking a prey item generated a pop up box stating whether they had made a correct or incorrect decision; for all other subjects, feedback was delayed, and information on the prey they had attacked was provided on a new screen only after pressing the “Next” button. A running score of a subject’s progress for both survey types was present at the top of each screen. On attacking a mimic the subject gained a point, and on attacking a model a point was deducted (where benefits = costs = 1). Therefore, I limited my study of SDT to systems in which costs of attacking unpalatable prey and the benefits of attacking palatable prey were identical.

5.3.7 *Testing Background*

Because, in natural conditions, a foraging herbivore is unlikely to search for prey items against a plain background, the ability to search for mimics with a “disruptive” background was tested on a subsample of subjects (Background Survey) (Fig. 5.1). Using a coloured background image of a natural bush setting as a “disruptive” backdrop (Fig. 5.4), natural images of leaves (images containing both traits of leaf colour and shape) were presented with the same set up as the surveys using the multi-dimensional coloured leaves containing no disruptive backdrop in order to compare any effects.

Fig. 5.4. An example of a screen from the *Similar* survey with leaves on a “disruptive” background image.

5.3.8 *Subjects’ use of traits*

Subjects were asked at the end of their survey, “What, if any, strategy did you use for identifying Species A (mimics) from Species B (models)?” as an open ended question. These strategies were then categorised. For the leaf silhouettes, the categories were: ‘shape only’, ‘size only’, and ‘size and shape’. For the coloured leaves there were seven categories: ‘colour only’, ‘shape only’, ‘size only’, ‘shape and size’, ‘colour and shape’, ‘colour and size’, and ‘all characteristics’.

5.3.9 *Statistical Analysis*

The proportions of mimics and models a subject attacked for each screen were used to calculate the SDT statistic A , (Zhang and Mueller 2005). A is the non-parametric version of the traditional SDT sensitivity index statistic d' . Sensitivity refers to how easy or hard a subject finds it to detect a signal (mimic) against background ‘noise’. The level of sensitivity is of interest as it can be used to compare how the different traits are used as signals by the subjects under different conditions, and therefore the potential impact on mimics. If a subject has a high “hit” (H) rate (selecting a mimic) and a low “false alarm” (FA) rate (selecting a

model), they are good at discriminating the presence of a mimic from the surrounding models, resulting in a high A value. As the FA rate increases relative to the H rate, subjects are becoming worse at detecting the presence of mimics from models, and the corresponding A value decreases. Thus, sensitivity provides a summary of the signal strength as perceived by subjects from the H and FA rates, and accounts for any strategy differences a subject may have when making trade-off decisions between the benefits of correct decisions versus the risks of making an incorrect decision during different tasks. A similar level of sensitivity can occur with varying H and FA rates in a given task based on the risks a signal receiver is willing to take. For example, a subject who chooses to take a high risk strategy by selecting lots of leaves and has a high H rate but also a high FA rate can have the same level of sensitivity as someone who is more cautious and has a low H rate but consequently also a lower FA rate. Therefore whilst accounting for the different strategies subjects may use, A correlates with the accuracy of the mimics signal to that of the model under the perceptual abilities of the subjects. A higher A value indicates the subjects were better at performing the discrimination task of identifying mimics from models, therefore A is used in this study as a measure of how well subjects “performed” at identifying mimics under different conditions. A is calculated with the following formula:

$$A = \begin{cases} \frac{3}{4} + \frac{H - FA}{4} - FA(1 - H) & \text{if } FA \leq 0.5 \leq H ; \\ \frac{3}{4} + \frac{H - FA}{4} - \frac{FA}{4H} & \text{if } FA \leq H < 0.5 ; \\ \frac{3}{4} + \frac{H - FA}{4} - \frac{1 - H}{4(1 - FA)} & \text{if } 0.5 < FA \leq H . \end{cases}$$

Possible effects on A of the ratio of models to mimics, of feedback and of leaf similarity grouping across the three dimensional traits were tested using an ANOVA. As subjects may use different strategies to attack mimics and models but still result in similar measures of A , a MANOVA was run on the arcsine-transformed proportions of mimics and models attacked. As the proportions of mimics and models attacked were not normally distributed, they were arcsine-transformed. A simple effects analysis was run to explore any significance within a statistical interaction between independent variables.

To test if the leaf trait characteristic that subjects reported they had used to identify mimics and models affected performance between the *Similar* and *Dissimilar* leaf groupings, an ANOVA was run on Box-Cox-transformed final scores. As subjects exposed to the trait of leaf colour only had few characteristics to use to help identify mimics and models, testing

was limited to subjects exposed to the trait leaf shape only and the combination of leaf colour and shape.

The number of independent variables within each ANOVA and MANOVA varied between each hypothesis being tested. For the difference between the *Similar* and *Dissimilar* grouped leaves, there were four variables: similarity grouping (*Similar* or *Dissimilar*), trait grouping (leaf colour only, leaf shape only, leaf colour and shape), feedback (instant or delayed), and ratio of mimics to models (1:3, 1:1, 3:1). To test the effect of a disruptive background there were three variables: background (disruptive or none), similarity grouping and ratio. For the remaining three hypotheses, only the trait grouping, feedback and ratio variables were included within the ANOVA and MANOVA as the *Similar* and *Dissimilar* surveys were run independent of each other.

A main assumption of SDT is that signal receivers (in this case the human subjects) have learnt the differences between mimics and models and will act accordingly. Because screens were randomly presented to subjects, the possible effects on a subject's performance of the order in which the screens were presented were tested using a Bonferroni post-hoc test in order to test if any learning was still occurring between screens. Only the first screen randomly presented to participants showed any significant difference from the remaining screens, therefore was removed from further analysis. All statistical analysis was performed on SPSS Statistics version 23 for Windows (IBM, Armonk, New York, USA).

5.4 RESULTS

A MANOVA was conducted on the overall mean proportions of mimics and models attacked on encounter (arcsine transformed), and for the SDT measure, *A*. There were several significant statistical interactions (Table 5.1). Below I discuss these results.

Table 5.1 Statistically significant MANOVA results between leaf groupings and within leaf groupings for the proportion of mimics and models selected by subjects, and subjects' level of sensitivity as measured by the SDT measure, *A*.

	df	df error	<i>F</i>	<i>P</i> -value
Between <i>Similar</i> and <i>Dissimilar</i> leaf groupings				
Mimics				
Trait	2	2523	30.16	< 0.01
Models				
Trait*Feedback	2	2523	7.34	< 0.01
<i>A</i>				
Trait	2	2509	35.45	< 0.01
Within <i>Dissimilar</i> leaf grouping				
Mimics				
Trait	2	1257	11.23	< 0.01
Feedback	2	1257	39.73	< 0.01
Models				
Trait	2	1257	44.66	< 0.01
Ratio	2	1257	3.03	0.05
<i>A</i>				
Trait	2	1227	20.53	< 0.01
Feedback	1	1227	7.60	0.01
Within <i>Similar</i> leaf grouping				
Mimics				
Trait*Feedback	2	1266	5.18	0.01
Trait*Ratio	4	1266	6.78	< 0.01
Models				
Trait*Feedback	2	1266	7.62	< 0.01
Trait*Ratio	4	1266	9.72	< 0.01
<i>A</i>				
Trait*Feedback	2	1230	5.31	0.01
Between a "disruptive" and plain background				
Mimics				
Background	1	1145	8.56	< 0.01
Models				
Background	1	1145	3.91	0.05
df, degrees of freedom; F, F values				

5.4.1 How the variation in leaf shape between mimics and models affects subjects' identification abilities

Subjects found it easier to identify mimics and avoid models when given leaf images that were most similar to one another (*Similar* leaf grouping), in comparison to those given the *Dissimilar* leaf group (Fig. 5.5). The greater ability of subjects to discriminate mimics whilst avoiding models correlated with a higher performance as measured by the signal detection theory measure of sensitivity, A , from subjects' within the *Similar* leaf group (Fig. 5.6).

However, there were differences in sensitivity depending on what trait or traits subjects' were using, and the type of feedback they were exposed to.

Fig. 5.5. The proportion of mimics (dark bars) and models (light bars) subjects selected when mimics and models were dissimilar (top row) and similar (bottom row) in leaf shape (*Dissimilar* and *Similar* leaf groupings) within the three trait types (columns) tested at the two types of feedback. Levels of statistical significance within each graph grouping represented by asterisks (* $p < 0.05$; ** $p < 0.01$). Statistical significance across leaf similarity grouping (horizontally) represented by letters ($p < 0.01$). Between leaf similarity groupings (vertically), all pairings statistically significant at $p < 0.01$, except: B-N, E-P, F-Q, and G-R. Data are means $\pm$ S.E.

Fig. 5.6. Subjects ability to differentiate between mimics and models as quantified by the signal detection theory measure of sensitivity statistic, A , between groupings of mimics and models that were dissimilar (top row) and similar (bottom row) in leaf shape (*Dissimilar* and *Similar* leaf groupings) within the three trait types (columns) tested under delayed feedback (dark bars) and instant feedback (light bars). A higher value equates to greater sensitivity in subjects. Levels of statistical significance within each graph grouping represented by asterisks (** $p < 0.01$). Statistical significance across leaf similarity grouping (horizontally) represented by letters ($p < 0.01$). Between leaf similarity groupings (vertically), all pairings statistically significant at $p < 0.01$, except: B-I, D-J, and E-K. Data are means $\pm$ S.E.

The subjects were better able to identify mimics, and to avoid the models in the *Similar* leaf groupings if they were given information on leaf colour as well as that on leaf shape (Fig. 5.5). Subjects' sensitivity when presented with the *Similar* leaf group was approximately 24% higher than when given *Dissimilar* group using the same trait combinations (Fig. 5.6). When colour was removed from leaf images (leaf shape only), subjects still found mimics easier to

identify from the *Similar* group, but models were only easier to avoid when feedback was delayed. The ability to avoid the models did not differ between the *Similar* and *Dissimilar* groupings when subjects were given instant feedback (Fig. 5.5). Delaying the feedback led to a 26% increase in sensitivity with the *Similar* grouped leaves in comparison with the *Dissimilar* grouped leaves. However, the lack of difference in ability to avoid models when feedback was instant resulted in no difference in sensitivity between the *Similar* and *Dissimilar* groups under instant feedback (Fig. 5.6). When given information only on leaf colour, subject's ability to identify mimics and to avoid models did not differ between the *Similar* and *Dissimilar* groups, apart from a very small improvement in identifying mimics from the *Similar* group with delayed feedback (Fig. 5.5). However, it was not significant enough to influence overall sensitivity as there was no difference between the *Similar* and *Dissimilar* groups when subjects were using only leaf colour (Fig. 5.6).

5.4.2 How leaf traits affect subjects' identification abilities

Subjects found leaf colour was the least informative trait (Fig. 5.5); subjects given this information only were the least sensitive (Fig. 5.6). Leaf shape enabled subjects to distinguish between mimics and models, but when leaf shape was combined with colour and when leaf images were most similar to one another, mimics and models became the most identifiable (Fig. 5.5) and sensitivity was the highest (Fig. 5.6).

Subjects who based their selection on information on leaf colour were the least able to discriminate between model and mimic. Subjects who used leaf shape were better at identifying mimics and better at avoiding models compared to those who used leaf colour (Fig. 5.5). This resulted in a 34% increased sensitivity for subjects given the *Similar* leaves, and an 18% increased sensitivity for those given the *Dissimilar* leaves, in comparison with subjects who used leaf colour (Fig. 5.6). Although the addition of colour to leaf shape increased subjects' abilities to identify mimics from among both the *Similar* and *Dissimilar* leaf groupings, it did not have a significant effect on model identification. For the *Similar* leaves, the subjects tended to avoid models only when presented with information on leaf shape and colour, and when given instant feedback (Fig. 5.5) or when the frequency of mimics was comparable to or higher (3:1) than that of the models (Fig. 5.8B). When given information on both leaf colour and shape, sensitivity in the *Similar* group increased by 13% in comparison with subjects who were only given leaf shape (Fig. 5.6). For the *Dissimilar* leaf group, subjects were no better at avoiding models or identifying mimics with the addition

of colour to leaf shape than those who used only leaf shape. (Fig. 5.5). Therefore, with the addition of colour within the *Dissimilar* leaf group, there was no change in sensitivity over just leaf shape (Fig. 5.6).

5.4.3 *How the amount of variation in leaf shape between mimics and models affects subjects' use of leaf trait characteristics for identification*

Differences in leaf shape, colour and size, were used either individually or in combination, by the subjects to distinguish between models and mimics. When presented with information only on leaf shape, there were only two characteristics subjects were able to use (size, and/or shape).

In the *Dissimilar* group of leaves, mimics differed most from models in their leaf size and size-related shape characteristics as described by PC1 (See *Prey* section in Methods). However, when given images of leaves from the *Dissimilar* group, subjects who said they used leaf shape, rather than leaf size, to distinguish between mimics and models scored the highest. A perfect score if every mimic was selected and every model was avoided would equal 99 (99 mimics across 8 screens). When using leaf shape only, those that used shape characteristics only scored higher (39.4 ± 4.2 S.E) than those that used both size and shape characteristics (29.9 ± 4.5 S.E) or just size characteristics (16.3 ± 5.7 S.E). When the images had information on both leaf colour and shape, shape again provided subjects with the highest scores, either by itself (58.0 ± 4.0 S.E) or in combination with colour (55.5 ± 4.5 S.E), with subjects who used colour alone scoring the lowest (17.8 ± 4.6 S.E) (Fig. 5.7A).

Leaf size and size-related shape characteristics differed less between model and mimic in the *Similar* than in the *Dissimilar* groups and correlated with greater subject sensitivity (Figs. 5.5 and 5.6). Subjects in the *Similar* grouping still identified size as a useful characteristic for discriminating between model and mimic; they gained the highest scores when using a combination of leaf size and shape characteristics (49.3 ± 4.5 S.E). However, subjects who used size only characteristics resulted in the lowest scores (28.0 ± 3.4 S.E). When given the additional information on leaf colour, shape still the most important leaf trait, correlating with the highest scores. Subjects who identified leaves using shape either by itself or in combination with the other characteristics scored higher (scores ranged between 62.4 ± 6.9 S.E to 74.5 ± 4.0 S.E) than subjects who used either size characteristic by itself or without the addition of shape characteristics (scores ranged between 36.8 ± 11.4 S.E and 47.5 ± 11.9 S.E) (Fig. 5.7B).

Fig. 5.7. The leaf trait characteristics subjects used to identify mimics and models. Traits used affected their final score (left vertical axis), however the proportion of subjects that used each trait varied (right vertical axis). ANOVA showed a statistically significant difference between characteristics used and the trait: (A) leaf shape only, within the *Dissimilar* leaf group (dark bars) ($F(2,70) = 5.36$, $p = 0.01$) and the *Similar* leaf group (light bars) ($F(2,70) = 3.89$, $p = 0.03$), and (B) when leaf shape and colour were combined, within the *Dissimilar* leaf group (dark bars) ($F(5,80) = 4.39$, $p < 0.01$), and the *Similar* leaf group (light bars) ($F(5,80) = 5.54$, $p < 0.01$). A perfect score where every mimic was selected and every model was avoided would equal 99. Lines indicate the proportion of participants who identified using any given leaf trait characteristic (*Dissimilar*: dark bars, *Similar*: light bars). Asterisks indicate statistical significance between scores of *Similar* and *Dissimilar* pairs (* $p < 0.05$; ** $p < 0.01$). Letters refer to statistical significant differences in scores between characteristics used within a leaf grouping ($p < 0.05$). Data are mean $\pm$ S.E.

The higher sensitivity of subjects given the *Similar* leaf groups, as compared with those given the *Dissimilar* ones, appears to be correlated to the use of different leaf trait characteristics the subjects identified to discriminate between mimics and models. Subjects from the *Dissimilar* group, where size and size-related shape characteristics were the most pronounced, were more likely to use size to identify mimics and to avoid models. Conversely, the *Similar* grouped subjects, who were better at identifying mimics and avoiding models (Fig. 5.5), indicated that they were more likely to use shape characteristics which were correlated with higher scores (Figs. 5.7A and B).

5.4.4 *How did feedback affect subjects' identification abilities?*

Overall, subjects found it easier to identify mimics when given instant feedback, irrespective of their leaf groupings or the leaf trait information presented to them. Feedback had little effect on their ability to identify models, however, except for those given the *Similar* leaf grouping and presented with the trait of leaf shape, where subjects better avoided models when given delayed feedback (Fig. 5.5). Subjects' sensitivity was slightly higher with instant feedback than with delayed feedback; when presented with information on both leaf shape and colour, this increase was 3% for the *Similar* leaf grouping and 7% for the *Dissimilar* leaf grouping. When presented with information on leaf shape only the increase was 14% for the *Dissimilar* leaf grouping. In contrast, for subjects given information on leaf shape only within the *Similar* leaf grouping, sensitivity was actually greater by 9% when feedback was delayed (Fig. 5.6); this was attributable to subjects being significantly better at avoiding models under delayed feedback than identifying mimics under instant feedback. When subjects were presented with the trait of colour only, feedback had little impact except within the *Dissimilar* leaf grouping where mimics were easier to identify under instant feedback (Fig. 5.5), but this was not significant enough to affect sensitivity (Fig. 5.6).

5.4.5 *How does the ratio of models to mimics affect subjects' identification ability?*

The ratio of mimics to models had very little effect on how subjects identified mimics or avoided models when leaf images were most dissimilar to one another (Fig. 5.8A). The only statistically significant difference occurred for subjects given the *Dissimilar* leaves, who found mimics harder to identify when they were at the highest frequency relative to the models (3:1).

Fig. 5.8. The proportion of mimics (dark bars) and models (light bars) subjects selected when mimics and models were (A) dissimilar in leaf shape (*Dissimilar* leaf grouping), and (B) similar in leaf shape (*Similar* leaf grouping) across the three ratios (3:1, 1:1, 1:3) of mimics to models within the three trait types tested (leaf shape and colour, leaf shape only, and colour only). The first number in the ratio mix equates to the number of mimics, and the second number equates to models. Letters indicate statistical significance ($p < 0.05$). Data are means $\pm$ S.E.

When subjects were given leaf images from the *Similar* group, when the trait tested was leaf shape only, models became progressively easier to identify as the ratio of mimics to models decreased. With the addition of information on leaf colour however, mimics became progressively harder to identify as the ratio of mimics to models decreased (Fig. 5.8B). With no variation between subjects' ability to avoid mimics across the three ratios when the trait tested was leaf shape only, subjects' ability at avoiding models increased their sensitivity. Subjects' sensitivity was 6-7% lower when given a 3:1 ratio of mimics to models than when given 1:1 and 1:3 ratios. When the trait tested was leaf shape and colour, subjects' sensitivity was associated with their ability to identify mimics rather than to reject models. Overall though, there was no strong pattern to indicate that the ratio of mimics to models influenced subjects' ability to discriminate mimics from models (Fig. 5.9).

Fig. 5.9. Subjects' ability to differentiate between mimics and models as quantified by the signal detection theory measure of sensitivity statistic, A , from between groupings of mimics and models that were similar in leaf shape (*Similar* leaf grouping), across the three ratios of mimics to models, within the three leaf trait types tested. A higher value equates to greater sensitivity in subjects. Letters indicate statistical significance between treatments ($p < 0.05$). Data are means $\pm$ S.E.

5.4.6 How does the background affect subjects' identification ability?

Subjects found it slightly harder to identify mimics when presented with models among a “disruptive” background. However, *Dissimilar* grouped models were easier to identify with a “disruptive” background, whilst there was no difference between the two background types when identifying *Similar* grouped models (Fig. 5.10A). Due to mimics being easier to identify with no background, the presence of a “disruptive” background image resulted in a small but significant decrease in subjects' sensitivity by 5% (Fig. 5.10B).

Fig. 5.10. (A) The proportion of mimics (dark bars) and models (light bars) subjects selected when leaves were presented on a plain white background and a “disruptive” background. Mimics and models were dissimilar in leaf shape (*Dissimilar* leaf grouping) and similar in leaf shape (*Similar* leaf grouping). Letters indicate statistical significance between treatments and pairings ($p < 0.05$). (B) Subjects ability to differentiate between mimics and models as quantified by the signal detection statistic measure of sensitivity statistic, A , between groupings of mimics and models that are presented on a “disruptive” background (dark bar) and on a plain white background (light bar). Asterisks indicate statistically significant difference between treatments (** $p < 0.01$). Data are means $\pm$ S.E.

5.5 DISCUSSION

There were several key findings from this study into the use of leaf traits for discrimination between the two visually similar plant species *Alseuosmia pusilla* (purported mimic) and *Pseudowintera colorata* (purported model), using human subjects as surrogate herbivores. (i) *A. pusilla* and *P. colorata* that were similar in 93.7% of the total shared leaf shape variation were easier to discriminate between than those that were dissimilar (Figs. 5.5 and 5.6). When *A. pusilla* and *P. colorata* were of similar shape, subjects focused on differences in leaf shape characteristics to discriminate between leaves. When the two species were dissimilar in leaf shape, subjects were distracted by size differences between leaves, even though size was not a reliable distinguishing trait (Fig. 5.7). (ii) Of the leaf traits used to distinguish *A. pusilla* from *P. colorata*, colour alone is not a reliable trait, whilst leaf shape is the most important

trait. However, when combined, leaf colour potentiates leaf shape as a cue or signal, increasing subjects ability to distinguish *A. pusilla* from *P. colorata*, but only when leaves were of the same size (Figs. 5.5 and 5.6). (iii) Although *A. pusilla* became easier to discriminate with the addition of leaf colour to leaf shape when the two species were of similar leaf shape, this effect was lost when they were dissimilar in leaf shape (Figs. 5.5 and 5.6). (iv) Instant feedback generally increased subjects' sensitivity (Fig. 5.6) making it easier for subjects to discriminate *A. pusilla* from *P. colorata* (Fig. 5.5). (v) Only within *A. pusilla* and *P. colorata* that were similar in leaf shape was there any support for the frequency-dependency hypothesis that predicts that mimics become harder to discriminate as the proportion of models increases. This was only the case when subjects were presented images of leaves that were also in colour (Fig. 5.8). (vi) A “disruptive” background makes it harder for subjects to discriminate *A. pusilla* from *P. colorata* (Figs. 5.10A and B). Below I discuss the significance of these results for Batesian mimicry in plants.

The leaves of *A. pusilla* share a remarkable degree of similarity in both leaf colour and leaf shape to those of *P. colorata* (Yager et al. 2016), and it is hypothesised that at locations where the two plant species co-occur, *A. pusilla* may escape predation by effectively functioning as a Batesian mimic of *P. colorata* (Dawson 1988, Atkinson and Greenwood 1989). In order for Batesian mimicry to evolve, the accuracy in similarity of the shared trait or traits between the undefended species (mimic) to that of the defended species (model) must be at a level that decreases the likelihood of the mimic being attacked (Ruxton et al. 2004). To date no studies have provided quantifiable support for the existence of visual mimicry within plants (Schaefer and Ruxton 2009, 2011), yet there are a number of studies that have documented many cases of uncanny visual similarities between defended and undefended sympatric species (Lev-Yadun 2016). How human subjects identify *A. pusilla* and *P. colorata* leaves provides insight into how the leaf traits of an undefended species may evolve to mimic those of another defended species, therefore acting as cues or signals of palatability.

This study highlights how different leaf traits may interact when discriminating between species. Leaf size was an uninformative trait for species discrimination (Fig. 5.7). Indeed, when *A. pusilla* and *P. colorata* varied in leaf size, subjects' sensitivity was reduced (Fig. 5.6), and size appeared to act as a distraction, with both *A. pusilla* and *P. colorata* becoming harder to discriminate (Fig. 5.5). Although subjects were biased towards leaf size in this study, in general human subjects tend to ignore size as a trait in discrimination tasks, even if

it is an informative trait (Ashby and Maddox 1990, Kikuchi et al. 2015), unless differences between mimic and model are extreme (Kikuchi et al. 2015). When size differences are extreme, human subjects' focus may be innately drawn to this trait as it is the most recognisable difference. Whether leaves that were large or small were more likely to be selected by subjects was not tested in this study, but this may highlight the need to account for a herbivores innate trait preferences or biases in future studies.

Leaf shape recognition can be important for both invertebrates and vertebrates seeking out potential food sources (Rausher 1978, Parsons et al. 2006, Dell'aglio et al. 2016). Of the two individual traits in this experiment, subjects were most sensitive to leaf shape (Fig. 5.6), and it was the most important individual trait for *A. pusilla* discrimination (Fig. 5.5). When *A. pusilla* and *P. colorata* leaves were of similar size and most alike in 93.7% of the total shape variation, subjects were more sensitive to signals or cues (Fig. 5.6), and better at discriminating *A. pusilla* (Fig. 5.5). Once leaf size was no longer a distraction for subjects, it is possible that they focussed their attention on the remaining 6.7% of shape variation for identifiable differences that could act as cues or signals.

Many herbivorous animals, including mammals and insects also utilize colour vision when foraging for food (Birgersson et al. 2001, Griffiths et al. 2010, Skelhorn et al. 2010). Leaf colour, however, was not useful as a cue or signal (Fig. 5.6) as evidenced by subjects being just as likely to select a *P. colorata* as they were an *A. pusilla* (Fig. 5.5). Although leaf colour alone had no effect on *A. pusilla* or *P. colorata* discriminability, when combined with leaf shape it did have a potentiating effect, improving subjects' cue or signal sensitivity (Fig. 5.6) and discrimination abilities of *A. pusilla*. This effect was lost, however, when *A. pusilla* and *P. colorata* varied in size (Fig. 5.5). This suggests that leaf colour can increase the probability of detection and/or decreases the detection threshold, thereby increasing the overall conspicuousness of the leaf shape cue or signal, in line with the "amplifier hypothesis". It may also be possible that these results indicate evidence for the "alerting hypothesis", where leaf colour draws attention to the more informative leaf shape features. However, the only research supporting this hypothesis to date has come from multiple modalities e.g. visual and auditory cues (Sumby and Pollack 1954), tactile and visual cues (Spence et al. 1998, Macaluso et al. 2000), or vibration and visual cues (Hebets 2005). While colour vision could aid in a herbivores ability to discriminate *A. pusilla*, the size of a leaf will be ambiguous depending on its distance from the herbivore. Leaf morphology also varies throughout ontogeny and often as a response to exposed biotic and abiotic conditions (Nicotra et al.

2011). Therefore, a herbivore would likely need to discriminate between a range of leaf sizes both within and between species.

A foraging herbivore would most likely encounter potential food sources in succession rather than simultaneously, it would therefore rely on memory to associate leaf morphology with unpalatability. The accuracy of discriminating between successively viewed objects decreases rapidly, owing to inefficiencies in coding and retrieving of memories, as shown both in humans (Uchikawa and Ikeda 1981) and honeybees (Dyer and Neumeyer 2005). However, discrimination abilities can be improved when associations of palatability are regularly reinforced (Blough 1959). If a herbivore's ability to accurately discriminate between visually ambiguous food items, such as in potential mimicry cases, decreases over time due to degradation of memories, shorter feedback responses when eating food items from pre-ingestive cues such as taste or smell rather than slower acting post-ingestive feedback would result in higher discriminability accuracy on subsequent choices. Knowing that a just eaten food item is desirable quickly, would allow for the herbivore to continue to browse or graze on that food item without delay, enabling stronger associations of palatability. When feedback was instant, subjects were generally more sensitive to cues or signals (Fig. 5.6) and were better at discriminating *A. pusilla* rather than when feedback was delayed. But interestingly, neither type of feedback generally had an effect on the discriminability of *P. colorata* (Fig. 5.5). Therefore, a model that provides slow feedback on its palatability would likely increase the uncertainty of subsequent discrimination tasks between potential models and mimics, benefiting a mimic.

There was some evidence that as the proportion of models decreased, the protection of mimics through predator deception increased, as predicted under the frequency-dependency hypothesis. However, this was the case only when leaves were of similar size and under conditions where *A. pusilla* and *P. colorata* were easiest to discriminate (when leaf colour and shape traits were combined) (Figs. 5.8A and B). In all other cases under the conditions where subjects found it harder to discriminate *A. pusilla* (Fig. 5.5), there was no evidence that *A. pusilla* were any harder to discriminate when there were proportionately more *P. colorata*. Thus, only when subjects were the most confident in identifying *A. pusilla* did *A. pusilla* gain protection as the proportion of *P. colorata* increased. In all other circumstances these results are similar to those of McGuire et al. (2006) who interpreted the data as indicating that subjects were "over-analysing the problem they faced by adopting provisional rules in the hope of maximising their scores". In this study however, apart from conditions when *A.*

pusilla and *P. colorata* leaves were coloured and of similar shape, the proportion of *A. pusilla* selected across the three ratios was relatively low; either close to, or below, 50%. And in almost all cases, the proportion of *A. pusilla* selected was lower than any proportion selected within the three ratios of the similar shaped coloured leaves (Fig. 5.8). It is likely that irrespective of how many there are, *A. pusilla* leaves under these conditions are at some form of discriminatory threshold.

In the natural environment, cryptic or camouflaged prey may or may not be present at any given location, therefore predators do not know where to focus their attention. In this experiment, because of the way the survey was set up, the layout was identical on each screen, although *A. pusilla* and *P. colorata* were randomly distributed within them; thus, the subjects were likely to know where to focus their attention. Therefore, rather than testing for effects of increased crypticity, this experiment is actually testing how a visually complicated background affects the discriminability of shapes in a foreground similar to the heterogeneous habitat of plants, especially that of a typical habitat of *A. pusilla* and *P. colorata* as shown in Fig. 5.4. Visual camouflage can cover a number of strategies that aid in concealment and the prevention of detection and recognition (Stevens and Merilaita 2009a), including background complexity (Dimitrova and Merilaita 2012, 2014, Xiao and Cuthill 2016). For both birds and humans, the number of lines and edges in the visual background, referred to in previous studies as “congestion”, has a significant impact on detectability of prey (Xiao and Cuthill 2016). When *A. pusilla* and *P. colorata* leaves were presented to subjects on a background image of a natural bush setting, subjects’ sensitivity to cues or signals decreased (Fig. 5.10B). This was mostly due to *A. pusilla* becoming slightly harder to discriminate (although *P. colorata* within the *Dissimilar* group became slightly easier to discriminate) (Fig. 5.10A).

Plants are sessile organisms that vary in degrees of palatability for any particular herbivore. They are often surrounded by other plant species whose leaves vary in colouration, shape and size as well as overall structural morphology. This would have a twofold effect: (i) the greater number of surrounding and possible alternative food sources creates a visually complex environment in which to identify food, and (ii) it would be easier to identify alternative palatable food that is visually distinct from unpalatable food rather than those that are visually ambiguous. As the abundance of alternative prey items increases, predators are more likely to attack them and ignore ambiguous prey, reducing selection on the accuracy on a Batesian mimic (Lindström et al. 2004). Therefore, as mimics becoming slightly more cryptic

due to the visually disruptive background and as the leaves of any mimics and models will vary in size, if there is an abundance of alternative potential food plants available that are not as visually ambiguous as the mimic, then even a poor mimic would be protected from the herbivore.

Human sensitivity to signals as measured by A , was overall driven by subjects' ability to discriminate *A. pusilla* rather than *P. colorata*. This bias towards *A. pusilla* may be an effect of setting the costs and benefits equal (benefits = costs = 1). Whilst it is hard to estimate the true costs and benefits to a herbivore in a natural system, further studies should incorporate the effect of increasing the incentives (benefits) versus the increase in discentives (costs) to see how this may influence subjects discrimination focus. An increase in costs in theory should lead to a more conservative approach taken than seen in this study whilst an increase in benefits should provide a higher incentive to attack. This may influence how subjects are able to discriminate mimics as the frequency of mimics to models varies, more in line with the frequency-dependent hypothesis than what was found in this study.

In conclusion, this study demonstrates how leaf shape can be an informative trait used by potential herbivores for species discrimination, whilst other leaf traits may either enhance or distract within this process. However, exactly how may vary depending on the species of herbivore. It also highlights the importance of the multitude of other conditions a herbivore faces when trying to undertake discrimination tasks. Leaf shape, at least for humans, is the most informative leaf trait for discriminating between *A. pusilla* and *P. colorata*. And although leaf colour improved cue or signal sensitivity, when a herbivore is presented with a range of leaf sizes, its ability to correctly discriminate *A. pusilla* decreases. In a natural environment, the discriminability of *A. pusilla* would be further reduced given the complexity of the visual background on which leaves must be discriminated against. Modelling of selective pressure and experiments in Batesian mimicry suggest that predators mistaking models for mimics should drive the mimetic trait in models to evolve away (McGuire et al. 2006, Franks et al. 2009). Interestingly, whilst fast feedback on subjects' choices increased subjects' discrimination abilities of *A. pusilla*, it had little effect on their ability to discriminate *P. colorata*. Feedback seemed to affect how accurately subjects were able to remember discriminable characteristics of the mimic rather than the model. Therefore there may be little selective pressure for a model to provide faster feedback on its palatability to a herbivore as this would have little effect on the likelihood of models being attacked.

As there is so much shape and colour variation between leaves, the ability to replicate discrimination tasks using fresh plant material would have been very difficult to do to the level that was made possible through the use of computers and humans as surrogate herbivores. This study, like others that use human surrogates, all tested how discriminatory tasks were performed on essentially simple shapes and colours. Where this study differs is that I applied these discriminatory tasks to shapes and colours present in the natural world. In doing so, I have highlighted in plants how under different natural conditions that a possible mimic can become harder to discriminate from its model, and therefore relevant for the evolution of Batesian mimicry. The ability to quickly train large numbers of humans is a huge advantage compared with other animals. However, for these results to be applicable to a particular herbivore, as with other human surrogate studies, cognitive and sensory abilities required for the level of the discriminatory task would have to be comparable with humans.

Chapter 6: General Discussion

6.1 INTRODUCTION

Brown and Lawton (1991) proposed over a quarter of a century ago, that the diverse morphologies we see in plant leaves may be, in part, a response to herbivory pressure. They identified mimicry between plant species as one way in which herbivores may have influenced the size and shape of leaves. As a defensive strategy, Batesian mimicry is a relatively common phenomenon amongst animals, but until I completed the research for chapter four, it had yet to be definitively demonstrated to function in plants. The Batesian mimicry hypothesis has been invoked in several studies where a similarity in leaf shape and colouration has been observed between two or more plant species, but there has been no empirical evidence that this similarity dupes a shared herbivore and correlates with increased fitness for the mimic. In my thesis, I tested whether Batesian mimicry could be responsible for the perceived similarities in leaf shape and colouration between the chemically defended *Pseudowintera colorata* and its purported mimic, *Alseuosmia pusilla*.

6.2 Novel discoveries from this thesis

This thesis presents the strongest empirical evidence to date in support of defensive Batesian mimicry complex in plants. My data satisfy the criteria for unequivocal proof of mimicry. That is: (i) both the mimic and a known model are attacked by the same herbivore; (ii) the herbivore is unable to distinguish between them; and (iii) this confusion increases the fitness of the mimic (Schaefer and Ruxton 2009, 2011).

In chapter two, I established a spatially explicit morphometric analysis and demonstrated that the conspicuous leaf shape of the defended *P. colorata* is replicated by the relatively undefended *A. pusilla* across its entire growing range. In doing so, this is the first study that I am aware of that has compared leaf shape between two species at such a “high resolution” across an environmental gradient. Using this same approach in chapter three, I demonstrated that in a population of *P. colorata* and *A. pusilla* exposed to high mammalian herbivory pressure, leaf shape variation is reduced in both focal species, but not in other sympatric species. This is consistent with Batesian mimicry, wherein increased herbivory pressure selects for a stronger signal in the distinctive characteristic of the defended plant, and through the selection for mimicry, variation in the mimic’s phenotype converges on the model’s phenotype. Additionally, when alternative palatable food is preferentially targeted, *P.*

colorata increased in abundance and *A. pusilla* followed suit proportionately. Invertebrate herbivory was estimated to be similar on both species at both sites.

In chapter four, I demonstrated that *A. pusilla* has a frequency-dependent protective relationship with *P. colorata* in the presence of red deer (*Cervus elaphus scoticus*), a relatively common introduced mammalian herbivore. I established that deer find *A. pusilla* more palatable than *P. colorata*, and that after eating a *P. colorata* individual, deer became reluctant to eat either *P. colorata* or *A. pusilla*. Although the two plants differ significantly in VOC emissions, deer were just as likely in the first instance to sample *A. pusilla* as they were *P. colorata*, and were, therefore, unable to use olfactory cues or to visually differentiate between the two species. Finally, as the abundance of *P. colorata* proportionately increased, herbivory damage to both species reduced overall, but more importantly, herbivory damage to individual *A. pusilla* reduced.

In chapter five, I investigated how leaf characteristics of *A. pusilla* and *P. colorata* might be used as cues to differentiate between the two species under different conditions. Humans, as surrogate herbivores, found leaf size a distracting characteristic, and foraged more effectively when *A. pusilla* and *P. colorata* individuals were most similar in 94% of their shared shape variation. The trait, leaf colour, whilst unreliable by itself, acted to potentiate the trait, leaf shape, as a signal or cue. Fast feedback on species palatability improved accuracy on discriminating *A. pusilla*, but neither fast nor slow feedback improved discriminability on *P. colorata*. Finally, *A. pusilla* leaves were harder to discriminate when presented on a “disruptive” backdrop.

6.3 The significance of establishing *Alseuosmia pusilla* as a Batesian mimic of *Pseudowintera colorata*

My study is the first to demonstrate unequivocally that a relatively undefended plant species acts as a Batesian mimic in the presence of a visually similar, well defended model. In doing so, I satisfied the required criteria for proof of Batesian mimicry in plants as highlighted by Schaefer and Ruxton (2009, 2011). This also provides support to Brown and Lawton’s (1991) hypothesis that herbivores are, in part, responsible for the diversity of leaf shape in plants. The matter of the signal receiver in this mimicry complex not having shared an evolutionary history with either plant species, does not, I feel, lessen the significance of this study, but is exciting in its own right. Further, in this study I addressed a number of fundamental

hypotheses and restrictions plants likely contend with in the evolution and/or maintenance of mimicry. Below I discuss these in regards to my data in greater detail.

6.3.1 Leaf shape similarity across the entire shared range

Schaefer and Ruxton (2009) proposed that successful plant mimicry requires that the mimic and model converge in the perceptual world of an approaching herbivore such that it cannot easily distinguish between the two. However, leaves exhibit morphological plasticity and there is often considerable intraspecific variation in leaf shape even in the same environment (see section 1.1.2). Therefore, the leaf shape, size and colour of a putative mimic must be demonstrated to indeed overlap with the ranges of those of the model, and that any morphological convergence is more evident between individuals where the two species co-occur. Additionally, for leaf shape to be effective as a visual deterrent, it would need to be sufficiently distinct from those of surrounding species.

Until I had completed the research in chapter two, although a number of studies had proposed mimicry (see section 1.6.1), no studies have fulfilled these requirements for a purported plant Batesian mimicry complex. However, one study published by Gianoli and Carrasco-Urra (2014), demonstrated that the leaf shape of the vine *Boquila trifoliolata*, changes its leaf shape in 11 leaf traits to match those of several of its known hosts. While an intriguing result, there was no attempt to address if this was a localised occurrence, if the morphological convergence is always evident between individuals every time the species co-occur, or how the vine leaf shape correlated with host shape under different environmental conditions.

In the above-mentioned study, as well as others that have attempted to address leaf shape differences using simple shape traits selected by the researchers, e.g. leaf length and width, there is a risk of attributing characteristics to mimicry that are not relevant to the intended signal receiver (e.g. eye-of-the-beholder mimicry; see section 1.4.3.2). By using a more robust technique of morphometric analysis, the human element is removed and all shape variation, perceived and unperceived, can be accounted for, allowing for an unbiased and true comparison of overall shape similarity. This is of particular importance, especially when the signal receiver is unknown.

My morphometric analysis demonstrates how a robust technique can be applied to compare leaf shape variation within and between a purported mimic and model species, and how leaf shape responds spatially. It is also a reliable way to demonstrate whether leaf shape is a distinct characteristic within the environment, independent of human perception.

6.3.2 Leaf shape variability when exposed to high and low herbivory pressure

The uniformity of warning signal is assumed to increase its efficacy (Müller 1879, Beatty et al. 2004, Rowland et al. 2007), yet variation in the strength of warning signals exists when there are trade-offs in associative fitness benefits and costs (see section 1.3.3). Likewise, mimics always experience selection to more closely resemble their models (Ruxton et al. 2004), yet imperfect mimicry is common. A number of hypotheses have been invoked to explain how imperfect mimicry can be maintained (see section 1.4.2).

My research in chapter three, is the first to address the response of leaf shape variation under increased herbivory pressure in the field. To date, studies have focused on how insect herbivores use leaf shape, demonstrating that leaf shape can influence the ovipositioning choice, consumption and performance, and act as a cue of a plants palatability (see section 1.5), but none, to the best of my knowledge, has attempted to address how leaf shape responds to herbivory pressure. The response of leaf shape variation between *P. colorata* and *A. pusilla* and abundance of the two species between the two study sites, present support for the relaxed selection hypothesis (see section 1.4.3.1). However, rather than an increase in accuracy of mimicry, which was not seen, it appears that herbivores selected for an increase in signal strength (decreased variation in leaf shape seen only in *P. colorata* and *A. pusilla*), possibly to increase the distinctiveness of leaf shape from surrounding palatable species.

6.3.3 Testing the Batesian mimicry hypothesis in plants

Unequivocal proof of defensive Batesian mimicry requires evidence that: (i) both the mimic and a known model are attacked by the same herbivore; (ii) the herbivore is unable to distinguish between them; and (iii) this confusion increases the fitness of the mimic. Until I completed my research for chapter four, no study had attempted to, or satisfied these three criteria in plants. Furthermore, the finding that deer became reluctant to eat again after having eaten a *P. colorata* provides quantitative support for Augner and Bernay's (1998) model for the evolution of Batesian mimicry in plants.

The putative signal receiver in the *A. pusilla*-*P. colorata* Batesian mimicry complex is the now extinct moa; a group consisting of several species of ratite mega-herbivores commonly invoked in hypotheses to explain unusual plant growth forms in the New Zealand flora. While it is not possible to test whether moa avoid *A. pusilla* in the presence of *P. colorata*,

studies into other unusual New Zealand native plant forms have substituted extant ratite species in feeding trials (Bond et al. 2004, Pollock et al. 2007). Therefore, performing feeding trials on emu or ostriches may provide indirect evidence for the moa co-evolutionary hypothesis. The results from chapter four support what was observed in chapter three in the field. However, whether or not deer are acting as substitute species for moa, or these results are an example of exaptive Batesian mimicry, both pose interesting areas of discussion.

In New Zealand, the role of introduced deer species functionally replacing moa (Caughley 1988, Batcheler 1989), is a controversial topic (Atkinson and Greenwood 1989, Forsyth et al. 2010, Lee et al. 2010, Wood et al. 2013). The role of substitute species is often highlighted in terms of providing a lost service that have a conservation benefit for other vulnerable species (Schlaepfer et al. 2011), although the benefits are argued to be often outweighed by the negatives (Vitule et al. 2012). The role of a substitute species in maintaining an adaptive function in other species does not appear to be addressed in research. Likewise, the evolution of traits within separate species that then get co-opted to act as signals that provide a fitness benefit through mimicry has not been studied yet either, and addressing how to prove this in most natural mimicry systems may be hard. However, these results are exciting as red deer, an introduced species now classified by many as a pest, react to the similarity between *A. pusilla* and *P. colorata* as predicted by Batesian mimicry theory. Therefore deer may be resurrecting an historic selection regime of extinct herbivores and leading to microevolution in plants.

6.4 Leaf traits as cues and signals, and the function of feedback and backdrop

How herbivores use plant traits as signals and cues, when they use them, and the factors involved in how they learn about them have not been well studied. To date, all we know about the use of signals comes from predator-prey foraging systems (Skelhorn et al. 2016). I modelled my study in chapter five on similar studies performed using humans as substitute predators (e.g. McGuire et al. 2006, Kikuchi et al. 2015). However, studies that use humans as surrogates have also exclusively only used computer generated prey to test how traits respond as signals. I wanted to test how images of real prey items with natural variation within traits could be used as signals.

6.4.1 Multidimensional signals in mimicry

Multidimensional signals are ubiquitous in communication, yet overall it is still rare for mimetic signals to be studied within a multidimensional framework (Gaskett 2011, Rowe and Halpin 2013, Dalziel et al. 2015). In my study I found evidence of a characteristic within a trait (leaf size), that acted on perceptual biases of the surrogate herbivores, decreasing their sensitivity to honest signals. In relation to mimicry, this is a functional requirement within the perceptual exploitation hypothesis (see section 1.4.4.1). Human subjects continued to use leaf size as a defining characteristic when it was an obvious difference between individual leaves, but not necessarily between models and mimics. As leaf morphology, including leaf size, is highly variable within and between species, a foraging herbivore is likely to be presented with a range of leaf sizes at any given time.

6.4.2 Multidimensional signals in leaves

My results from chapter five demonstrate that leaves can act as cues and signals. Humans, as surrogate herbivores, used leaf traits and were able to discriminate between *A. pusilla* and *P. colorata* to varying degrees under different conditions. Leaf colour by itself was not informative. However, when combined with leaf shape, the sensitivity of respondents was greater than those who used leaf shape alone. Although not uncommon within predator-prey signalling (e.g. Hasson 1991, Hebets and Uetz 1999), this is the only example from within plants of the amplifying hypothesis.

6.4.3 Feedback and backdrop

Herbivores rely on feedback when selecting food in order to make associative connections between nutrient quality and secondary metabolite investment within a plant. Studies into feedback have focused on how feedback from post-ingestive cues (e.g. satiation) calibrate the sensory experiences from pre-ingestive cues (e.g. taste, colour) (Provenza 1995, Villalba and Provenza 2000, Werner and Provenza 2011). I was interested to see if the speed in which feedback was provided could affect the ability of signal learning. I am unaware of any other study that has focused on the role of feedback speed on discriminability. Faster feedback correlated with increased discriminability of the rewarding species (*A. pusilla*), however the speed of feedback generally did not affect the ability of subjects to avoid the unpalatable species (*P. colorata*). This suggests that the speed of feedback that is provided by a defended species is not likely to be under selection with an associated warning signal. Therefore, feedback speed is of interest in mimicry systems where mimics are at an advantage when the

unpalatability of a model's defences is expressed slowly. For example, a feeling of unwellness from ingesting a secondary metabolite would benefit a mimic more than a powerful taste.

Detectability of prey against background has also been well studied within predator-prey systems (Ruxton et al. 2004), yet how background might affect a foraging herbivore's ability to detect food has received very little attention. The only other studies that have looked at the effect of background in plants have focused on colour matching of plants to background (Hernández et al. 2001, Fadzly et al. 2009, Burns 2010), or mentioned that variable leaf shape could blend into the background (Fadzly and Burns 2010). However, many plant habitats are associated with a number of plant species and are visually complex environments where herbivores must contend with a lot of signal noise. My study suggests that plants, by nature of their environment, are cryptic to some degree.

6.4.4 Plant-herbivore communication

My results from chapter five cannot be directly applied to a particular herbivore-plant interaction given that I used human surrogates. However, as there is good qualitative congruity found between the performance of humans and other animals in simple discriminatory tasks, it does demonstrate that leaf shape can act as a signal or cue. These results indicate why further research into plant-herbivore communication is important and that it could provide powerful insights into the functional significance of leaf morphology.

6.5 CONCLUSIONS

The diversity of leaf shapes and sizes is vast. Leaf morphology and colouration can be partially attributed as a physiological response to abiotic and biotic stresses plants face (see section 1.1). However, as reviews on the functional significance of leaf shape all highlight, there is a need for further investigation into the role of herbivores (Tsukaya 2005, Kidner and Umbreen 2010, Nicotra et al. 2011, Dkhar and Pareek 2014). My thesis provides further support to the few studies that demonstrate that leaf shape, size and colour are under selection by herbivores. In chapter two, I demonstrate a robust method of studying leaf shape that is independent of human perceptual biases. In doing so I established the similarity in leaf shape between *A. pusilla* and *P. colorata*. In chapter three, I showed that variation in leaf shape decreased in natural populations of the two species when exposed to high numbers of mammalian herbivores. Both species were also in higher abundance as predicted under

Batesian mimicry. In chapter four, I satisfied the requirements for *A. pusilla* to act as a Batesian mimic of *P. colorata*. I demonstrated that deer found *A. pusilla* more palatable than *P. colorata*. Deer were equally likely to make first contact with either species, but as the proportion of *P. colorata* individuals increased relative to *A. pusilla*, browsing on *A. pusilla* individuals reduced. In chapter five, I showed that leaf traits can act as signals or cues. I also demonstrated that plant-herbivore communication requires further study as a greater understanding of how herbivores use plant cues and signals will aid our understanding of the functional significance of leaf shape. Our understanding of how herbivores identify palatable and unpalatable food, when they use particular cues or signals, and the factors involved in how they learn about them will also aid our understanding of how a plant may act as a Batesian mimic of another plant to avoid herbivory.

APPENDICIES

A. Additional information for Chapter 2

A.1. *Location of transect at Otaki Forks*

Plant material was collected along a 4.5km stretch of track in the Otaki Forks region of the Tararua Forest Park, New Zealand between the coordinates S40 53.30, E175 14.22 and S40 54.46, E175 15.37. The collection area covered the elevational range of 470 – 870 m (Fig. A.1). Plant locations were recorded using a Garmin 60 GPS (Olathe, USA; accuracy: $\pm$ 2-10 m).

Fig. A.1. (A) Otaki Forks field site location (red shaded box) located in the Tararua Forest Park, New Zealand. Scale bar = 5 km. (B) Study transect line (red) following Fields Track at Otaki Forks. Scale bar = 500 m.

A.2. Shape Analysis

When principal components (PC) 1-4 are plotted in two-dimensional space, there is overlap in the variation in leaf shape of the population of *Pseudowintera colorata* and *Alseuosmia pusilla* (Fig. A.2).

Fig. A.2. Two-dimensional graphs generated by ± 6 standard deviations from the mean leaf shape along each principal component (PC) representing leaves of *Pseudowintera colorata* (red crosses) and *Alseuosmia pusilla* (green open triangles) as single points. Principal component analysis reduced 98.1% of leaf shape variation between the two species into four PC's (PC1-4). PC1 accounted for 89.6% of total leaf shape variation, PC2: 4.1%, PC3: 3.4%, and PC4: 1%. Leaf shape variation between the two species is plotted along (A) PC1 and PC2; (B) PC2 and PC3; and (C) PC3 and PC4.

A.3. Shape Discrimination

When the discriminant analysis was restricted to *P. colorata* and *A. pusilla* only, discriminant analysis incorrectly classified 6% of *A. pusilla* as *P. colorata*. For *P. colorata*, 29.9% were misidentified as *A. pusilla* (Table A.1). Thus, a total of 18% *A. pusilla* and *P. colorata* were misclassified.

Table A.1. Percentage of correct identification of species by cross validated discriminant analysis using the leaf margin landmarks.

Original species group membership	Predicted species group membership based on leaf shape (%)	
	<i>A.p</i>	<i>P.c</i>
<i>Alseuosmia pusilla</i> (<i>A.p</i>)	94.0	6.0
<i>Pseudowintera colorata</i> (<i>P.c</i>)	29.9	70.1

B. Additional information for Chapter 3

B.1. Location of transect at Kapakapanui

Plant material was collected along a 3.5km stretch of track in the Kapakapanui region of the Tararua Forest Park, New Zealand between the coordinates S40 55.15, E175 08.14 and S40 54.66, E175 09.01. The collection area covered the elevational range of 517 – 715 m (Fig. B.1). Plant locations were recorded using a Garmin 60 GPS (Olathe, USA; accuracy: $\pm$ 2-10 m).

Fig. B.1. (A) Kapakapanui field site location (red shaded box) located in the Tararua Forest Park, New Zealand. Scale bar = 5 km. (B) Study transect line (red) following Kapakapanui hut track at Kapakapanui. Scale bar = 500 m.

B.2. Shape Analysis

When principal components (PC) 1-4 are plotted in two-dimensional space, the overlap in the variation in leaf shape of the population of *Pseudowintera colorata* and *Alseuosmia pusilla* can be demonstrated within and between the “High Herbivore” site and the “Low Herbivore” site (Fig. B.2).

Fig. B.2. Two-dimensional graphs generated by ± 6 standard deviations from the mean leaf shape along each principal component (PC) representing leaves from the “Low Herbivore” site: *Pseudowintera colorata* (red crosses) and *Alseuosmia pusilla* (green open triangles), and the “High Herbivore” site: *P. colorata* (light red squares) and *A. pusilla* (light green triangles) as single points. Principal component analysis reduced 97.7% of leaf shape variation between the two species into four PC’s (PC1-4). PC1 accounted for 87.6% of total leaf shape variation, PC2: 4.7%, PC3: 4.3%, and PC4: 1.1%. Leaf shape variation between the two species is plotted along (A) PC1 and PC2; (B) PC2 and PC3; and (C) PC3 and PC4.

C. Additional information for Chapter 4

C.1. Palatable control group species

Hoheria sexstylosa and *Griselinia littoralis* saplings, two known palatable tree species to red deer (*Cervus elaphus scoticus*) (Nugent et al. 1997, Forsyth et al. 2002), were tested in the

presence of *P. colorata* as control groups. The leaves of *H. sexstylosa* and *G. littoralis* are visually dissimilar in leaf shape (Fig. C.1), and all individuals of both species presented to deer in each feeding trial were of similar height.

Fig. C.1. Leaf morphology examples of, (A) *Griselinia littoralis* and (B) *Hoheria sexstylosa*, two known palatable tree species to red deer (*Cervus elaphus scoticus*), used in feeding trials as control groups. Scale bar = 20 mm.

D. Additional information for Chapter 5

D.1. Leaf selection for Similar and Dissimilar groups

Individual leaves were categorised as *Similar* or *Dissimilar* based on leaf shape characteristics only (not colour) from their principal component (PC) 1 and PC2 scores. PC1 and PC2 accounted for 93.7% of the total leaf shape variation. The *Similar* group comprised model and mimic leaves that lay within a 1 S.D. ellipse of the PC1 and PC2 mean leaf shape. That is, a screen presented to a subject contained images of *Pseudowintera colorata* and *Alseuosmia pusilla* leaves either from within the *P. colorata* ellipse, the *A. pusilla* ellipse, or the overlap of the *P. colorata* and *A. pusilla* ellipses (Fig. D.1). Each screen therefore contained leaves with minimal difference in PC1 and PC2 scores. The *Dissimilar* group was made up of leaves from outside the 1 S.D. ellipses of the mean leaf shapes, but for each screen, the presented leaves of mimics were matched such that there was maximum difference in PC1 and PC2 scores from the presented leaves of models. That is, if images of

P. colorata were sourced from PC1 scores with negative S.D. values and PC2 scores with positive S.D. values (e.g. top left quadrant of the graph in Fig. D1), then images of *A. pusilla* were sourced from PC1 scores with positive S.D. values and PC2 scores with negative S.D. values (e.g. lower right quadrant of the graph in Fig. D.1).

Fig. D.1. Images of leaves for the *Similar* and *Dissimilar* groups were selected based on their principal component (PC) 1 and PC2 scores. *Similar* grouped leaves were found within the 1 S.D. ellipses from the mean leaf shape of either species. *Dissimilar* grouped leaves were located from outside the 1 S.D. ellipses and from opposite quadrants from the PC1 and PC2 graph.

REFERENCES

- Abbott, K.R., and Sherratt, T.N. 2013. Optimal sampling and signal detection: Unifying models of attention and speed-accuracy trade-offs. *Behav. Ecol.* **24**(3): 605–616. doi:10.1093/beheco/art001.
- Agrawal, A.A. 1998. Induced responses to herbivory and increased plant performance. *Science* **279**(5354): 1201–1202. doi:10.1126/science.279.5354.1201.
- Agrawal, A.A. 2011. Current trends in the evolutionary ecology of plant defence. *Funct. Ecol.* **25**(2): 420–432. doi:10.1111/j.1365-2435.2010.01796.x.
- Agrawal, A.A., and Fishbein, M. 2006. Plant defense syndromes. *Ecology* **87**(7 (Supplement)): S132–S149. doi:10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2.
- Agrawal, A.A., Petschenka, G., Bingham, R.A., Weber, M.G., and Rasmann, S. 2012. Toxic cardenolides: chemical ecology and coevolution of specialized plant-herbivore interactions. *New Phytol.* **194**(1): 28–45. doi:10.1111/j.1469-8137.2011.04049.x.
- Aizen, M.A., Sabatino, M., and Tylianakis, J.M. 2012. Specialization and rarity predict nonrandom loss of interactions from mutualist networks. *Science*. **335**(6075): 1486–1489. doi:10.1126/science.1215320.
- Allen, R.B., Payton, I.J., and Knowlton, J.E. 1984. Effects of ungulates on structure and species composition in the Urewera forests as shown by exclosures. *N. Z. J. Ecol.* **7**: 119–130. Available from <http://www.jstor.org/stable/24052708>.
- Anderson, B., Johnson, S.D., and Carbutt, C. 2005. Exploitation of a specialized mutualism by a deceptive orchid. *Am. J. Bot.* **92**(8): 1342–1349. doi:10.3732/ajb.92.8.1342.
- Anne, P., Mawri, F., Gladstone, S., and Freeman, D.C. 1998. Is fluctuating asymmetry a reliable biomonitor of stress? A test using life history parameters in soybean. *Int. J. Plant Sci.* **159**(4): 559–565. doi:10.1086/297573.
- Anten, N.P.R., Alcalá-Herrera, R., Schieving, F., and Onoda, Y. 2010. Wind and mechanical stimuli differentially affect leaf traits in *Plantago major*. *New Phytol.* **188**(2): 554–564. doi:10.1111/j.1469-8137.2010.03379.x.
- Archetti, M. 2009a. Phylogenetic analysis reveals a scattered distribution of autumn colours. *Ann. Bot.* **103**(5): 703–713. doi:10.1093/aob/mcn259.

- Archetti, M. 2009b. Evidence from the domestication of apple for the maintenance of autumn colours by coevolution. *Proc. R. Soc. B Biol. Sci.* **276**(1667): 2575–2580. doi:10.1098/rspb.2009.0355.
- Archetti, M., and Brown, S.P. 2004. The coevolution theory of autumn colours. *Proc. R. Soc. B Biol. Sci.* **271**(1545): 1219–1223. doi:10.1098/rspb.2004.2728.
- Aronsson, M., and Gamberale-Stille, G. 2013. Evidence of signaling benefits to contrasting internal color boundaries in warning coloration. *Behav. Ecol.* **24**(2): 349–354. doi:10.1093/beheco/ars170.
- Asakawa, Y., Dawson, G.W., Griffiths, D.C., Lallemand, J.Y., Ley, S.V., Mori, K., Mudd, A., Pezechk-Leclaire, M., Pickett, J.A., Watanabe, H., Woodcock, C.M., and Zhong-Ning, Z. 1988. Activity of drimane antifeedants and related compounds against aphids, and comparative biological effects and chemical reactivity of (-) and (+) polygodial. *J. Chem. Ecol.* **14**(10): 1845–55. doi:10.1007/BF01013481.
- Ashby, F.G., and Maddox, W.T. 1990. Integrating information from separable psychological dimensions. *J. Exp. Psychol. Hum. Percept. Perform.* **16**(3): 598–612. doi:10.1037/0096-1523.16.3.598.
- Ashwell, K.W.S., and Scofield, R.P. 2008. Big birds and their brains: paleoneurology of the New Zealand moa. *Brain. Behav. Evol.* **71**(2): 151–166. doi:10.1159/000111461.
- Atkinson, I.A.E., and Greenwood, R.M. 1989. Relationships between moas and plants. *N. Z. J. Ecol.* **12**(2): 67–96. Available from <http://www.jstor.org/stable/24053251> [accessed 15 November 2011].
- Augner, M., and Bernays, E.A. 1998. Plant defence signals and Batesian mimicry. *Evol. Ecol.* **12**(6): 667–679. doi:10.1023/A:1006581415114.
- Balogh, A.C.V., Gamberale-Stille, G., Tullberg, B.S., and Leimar, O. 2010. Feature theory and the two-step hypothesis of Müllerian mimicry evolution. *Evolution* **64**(3): 810–822. doi:10.1111/j.1558-5646.2009.00852.x.
- Baraza, E., Villalba, J.J., and Provenza, F.D. 2005. Nutritional context influences preferences of lambs for foods with plant secondary metabolites. *Appl. Anim. Behav. Sci.* **92**(4): 293–305. doi:10.1016/j.applanim.2004.11.010.

- Barlow, B.A., and Wiens, D. 1977. Host-parasite resemblance in Australian mistletoes: the case for cryptic mimicry. *Evolution* **31**(1): 69–84. doi:10.2307/2407546.
- Barrett, S.H. 1983. Crop mimicry in weeds. *Econ. Bot.* **37**(3): 255–282. doi:10.1007/BF02858881.
- Barthélémy, D., and Caraglio, Y. 2007. Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Ann. Bot.* **99**(3): 375–407. doi: 10.1093/aob/mcl260.
- Batcheler, C.L. 1989. Moa browsing and vegetation formations, with particular reference to deciduous and poisonous plants. *N. Z. J. Ecol.* **12**(Suppl.): 57–65. Available from <http://www.jstor.org/stable/24053250>.
- Bates, H.W. 1862. Contributions to an insect fauna of the Amazon valley (Lepidoptera: *Heliconidae*). *Trans. Linn. Soc. London* **23**(3): 495–566. doi:10.1111/j.1096-3642.1860.tb00146.x
- Beatty, C.D., Beirinckx, K., and Sherratt, T.N. 2004. The evolution of Müllerian mimicry in multispecies communities. *Nature* **431**(7004): 63–66. doi:10.1038/nature02904.
- Berenbaum, M.R., and Miliczky, E. 1984. Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. *Am. Midl. Nat.* **111**(1): 64–68. doi:10.2307/2425543.
- Bernays, E.A., and Chapman, R.G. 1994. *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, New York. doi:10.1007/b102508.
- Birgersson, J., Alm, U., and Forkman, B. 2001. Colour vision in fallow deer: a behavioural study. *Anim. Behav.* **61**(2): 367–371. doi:doi.org/10.1006/anbe.2000.1603.
- Blick, R.A.J., Burns, K.C., and Moles, A.T. 2012. Predicting network topology of mistletoe-host interactions: do mistletoes really mimic their hosts? *Oikos* **121**(5): 761–771. doi:10.1111/j.1600-0706.2011.19854.x.
- Blough, D. 1959. Delayed matching in the pigeon. *J. Exp. Anal. Behav.* **2**(2): 151–160. doi:10.1901/jeab.1959.2-151.

- Bond, W.J., Lee, W.G., and Craine, J.M. 2004. Plant structural defences against browsing birds: a legacy of New Zealand's extinct moas. *Oikos* **104**(3): 500–508. doi:10.1111/j.0030-1299.2004.12720.x.
- Bond, W.J., and Silander, J.A. 2007. Springs and wire plants: anachronistic defences against Madagascar's extinct elephant birds. *Proc. R. Soc. B Biol. Sci.* **274**(1621): 1985–1992. doi:10.1098/rspb.2007.0414.
- Boyce, C.K. 2009. Seeing the forest with the leaves - clues to canopy placement from leaf fossil size and venation characteristics. *Geobiology* **7**(2): 192–9. doi:10.1111/j.1472-4669.2008.00176.x.
- Brower, J.V.Z. 1960. Experimental studies of mimicry. IV. The reactions of starlings to different proportions of models and mimics. *Am. Nat.* **94**(877): 271–282. doi:10.1086/282128.
- Brower, L.P., and Brower, J.V.Z. 1962. The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. *Ecology* **43**(1): 154–158. doi:10.2307/1932059.
- Brown, V.K., and Lawton, J.H. 1991. Herbivory and the evolution of leaf size and shape. *Philos. Trans. R. Soc. Biol. Sci.* **333**(1267): 265–272. doi:10.1098/rstb.1991.0076.
- Bunce, M., Worthy, T.H., Phillips, M.J., Holdaway, R.N., Willerslev, E., Haile, J., Shapiro, B., Scofield, R.P., Drummond, A., Kamp, P.J.J., and Cooper, A. 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. *Proc. Natl. Acad. Sci. U. S. A.* **106**(49): 20646–20651. doi:10.1073/pnas.0906660106.
- Burns, K.C. 2010. Is crypsis a common defensive strategy in plants? Speculation on signal deception in the New Zealand flora. *Plant Signal. Behav.* **5**(1): 9–13. doi:10.4161/psb.5.1.10236.
- Burns, K.C., and Dawson, J.W. 2009. Heteroblasty on Chatham Island: a comparison with New Zealand and New Caledonia. *N. Z. J. Ecol.* **33**(2): 156–163. Available from <http://www.jstor.org/stable/24060618> [accessed 15 November 2011].
- Cambie, R.C., and Parnell, J.C. 1970. New Zealand phytochemical survey. 8. Constituents of some New Zealand plants. *New Zeal. J. Sci.* **13**(1): 108–116.

- Campitelli, B.E., Simonsen, A.K., Rico Wolf, A., Manson, J.S., and Stinchcombe, J.R. 2008a. Leaf shape variation and herbivore consumption and performance: a case study with *Ipomoea hederacea* and three generalists. *Arthropod. Plant. Interact.* **2**(1): 9–19. doi:10.1007/s11829-007-9028-x.
- Campitelli, B.E., Stehlik, I., and Stinchcombe, J.R. 2008b. Leaf variegation is associated with reduced herbivore damage in *Hydrophyllum virginianum*. *Botany-Botanique* **86**: 306–313. doi:10.1139/b07-139.
- Canyon, D.V., and Hill, G.J. 1997. Mistletoe host-resemblance: A study of herbivory, nitrogen and moisture in two Australian mistletoes and their host trees. *Austral Ecol.* **22**(4): 395–403. doi:10.1111/j.1442-9993.1997.tb00689.x.
- ten Cate, C., and Rowe, C. 2007. Biases in signal evolution: learning makes a difference. *Trends Ecol. Evol.* **22**(7): 380–387. doi:10.1016/j.tree.2007.03.006.
- Caughley, G. 1988. Plant-herbivore interactions. *In* Seminar 2000: The Future of New Zealand's wild animals? Proceedings of a national seminar organised by the New Zealand Deerstalkers Association. pp. 51–52.
- Charnov, E.L. 1976. Optimal foraging: attack strategy of a mantid. *Am. Nat.* **110**(971): 141–151. doi:10.1086/283054.
- Chittka, L., and Osorio, D. 2007. Cognitive dimensions of predator responses to imperfect mimicry. *PLoS Biol.* **5**(12): 2754–2758. doi:10.1371/journal.pbio.0050339.
- Chittka, L., and Raine, N.E. 2006. Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**(4): 428–435. doi:10.1016/j.pbi.2006.05.002.
- Christenhusz, M.J.M., and Byng, J.W. 2016. The number of known plants species in the world and its annual increase. *Phytotaxa.* **261**(3): 201–217. doi:10.11646/phytotaxa.261.3.1.
- Clauss, M., Steuer, P., Müller, D.W.H., Codron, D., and Hummel, J. 2013. Herbivory and body size: Allometries of diet quality and gastrointestinal physiology, and implications for herbivore ecology and dinosaur gigantism. *PLoS One* **8**(10). doi:10.1371/journal.pone.0068714.

- Coley, P.D., and Aide, T.M. 1989. Red coloration of tropical young leaves: a possible antifungal defence? *Journal. J. Trop. Ecol.* **5**(3): 293–300.
doi:10.1017/S0266467400003667.
- Cooney, L.J., van Klink, J.W., Hughes, N.M., Perry, N.B., Schaefer, H.M., Menzies, I.J., and Gould, K.S. 2012. Red leaf margins indicate increased polygodial content and function as visual signals to reduce herbivory in *Pseudowintera colorata*. *New Phytol.* **194**(2): 488–497. doi:10.1111/j.1469-8137.2012.04063.x.
- Corbett, R.E., and Grant, P.K. 1958. The volatile oil of *Pseudowintera colorata*. I. *J. Sci. Food Agric.* **9**(11): 733–739. doi:10.1002/jsfa.2740091108.
- Corlett, R.T. 2016. Plant diversity in a changing world: status, trends, and conservation needs. *Plant Divers.* **38**(1): 10–16. Elsevier Ltd. doi:10.1016/j.pld.2016.01.001.
- Da Costa, C.P., and Jones, C.M. 1971. Cucumber beetle resistance and mite susceptibility controlled by the Bitter gene in *Cucumis sativus* L. *Science* **172**(3988): 1145–1146. doi:10.1126/science.172.3988.1145.
- Cott, H.B. 1940. *Adaptive Coloration in Animals*. Methuen, London.
- Crawley, M.J. 1985. Reduction of oak fecundity by low-density herbivore populations. *Nature* **314**(6007): 163–164. doi:10.1038/314163a0.
- Crawley, M.J. 1989. Insect herbivores and plant population dynamics. *Annu. Rev. Entomol.* **34**(1): 531–562. doi:10.1146/annurev.en.34.010189.002531.
- Crooks, J.A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* **97**(2): 153–166. doi:10.1034/j.1600-0706.2002.970201.x.
- Cunningham, S.A., Summerhayes, B., and Westoby, M. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecol. Monogr.* **69**(4): 569–588. doi:10.1890/0012-9615(1999)069[0569:EDILSA]2.0.CO;2.
- Cuthill, I.C., and Bennett, A.T.D. 1993. Mimicry and the eye of the beholder. *Proc. R. Soc. B Biol. Sci.* **253**(1337): 203–204. doi:10.1098/rspb.1993.0103.
- Dalziell, A.H., Welbergen, J.A., Iqic, B., and Magrath, R.D. 2015. Avian vocal mimicry: a unified conceptual framework. *Biol. Rev.* **90**(2): 643–668. doi:10.1111/brv.12129.

- Darrow, H.E., Bannister, P., Burritt, D.J., and Jameson, P.E. 2001. The frost resistance of juvenile and adult forms of some heteroblastic New Zealand plants. *New Zeal. J. Bot.* **39**(2): 355–363. doi:10.1080/0028825X.2001.9512741.
- Darst, C.R., and Cummings, M.E. 2006. Predator learning favours mimicry of a less-toxic model in poison frogs. *Nature* **440**(7081): 208–11. doi:10.1038/nature04297.
- Darwin, C. 1871. *The Descent of Man and Selection in Relation to Sex*. J. Murray, London.
- Dawson, J.W. 1988. *Forest Vines to Snow Tussocks: The story of New Zealand plants*. Victoria University Press, Wellington.
- Dearing, M.D., Foley, W.J., and McLean, S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annu. Rev. Ecol. Evol. Syst.* **36**(1): 169–189. doi:10.1146/annurev.ecolsys.36.102003.152617.
- Dearing, M.D., Mangione, A.M., and Karasov, W.H. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. *Oecologia* **123**(3): 397–405. doi:10.1007/s004420051027.
- Dell’aglio, D.D., Losada, M.E., and Jiggins, C.D. 2016. Butterfly learning and the diversification of plant leaf shape. *Front. Ecol. Evol.* **4**: 81. doi:10.3389/FEVO.2016.00081.
- Department of Conservation. 2014. *Project Kaka: Tararua Nature Recovery. Progress report to January 2013*. Wellington. Available from <http://www.doc.govt.nz/Documents/conservation/land-and-freshwater/land/project-kaka-report-to-2013.pdf>.
- Dimitrova, M., and Merilaita, S. 2012. Prey pattern regularity and background complexity affect detectability of background-matching prey. *Behav. Ecol.* **23**(2): 384–390. doi:10.1093/beheco/arr201.
- Dimitrova, M., and Merilaita, S. 2014. Hide and seek: properties of prey and background patterns affect prey detection by blue tits. *Behav. Ecol.* **25**(2): 402–408. doi:10.1093/beheco/art130.

- Dimitrova, M., Stobbe, N., Schaefer, H.M., and Merliaita, S. 2009. Concealed by conspicuousness: distractive prey markings and backgrounds. *Proc. R. Soc. B Biol. Sci.* **276**(1663): 1905–1910. doi:10.1098/rspb.2009.0052.
- Dittrich, W., Gilbert, F., Green, P., McGregor, P., and Grewcock, D. 1993. Imperfect mimicry: a pigeon's perspective. *Proc. R. Soc. B Biol. Sci.* **251**(1332): 195–200. doi:10.1098/rspb.1993.0029.
- Dkhar, J., and Pareek, A. 2014. What determines a leaf's shape? *Evodevo* **5**(1): 47. doi:10.1186/2041-9139-5-47.
- Duncan, A.J., and Young, S.A. 2002. Can goats learn about foods through conditioned food aversions and preferences when multiple food options are simultaneously available? *J. Anim. Sci.* **80**(8): 2091–2098. doi:10.2527/2002.8082091x.
- Duncan, C.J., and Sheppard, P.M. 1963. Continuous and quantile theories of sensory discrimination. *Proc. R. Soc. B Biol. Sci.* **158**: 343–363.
- Duncan, C.J., and Sheppard, P.M. 1965. Sensory discrimination and its role in the evolution of Batesian mimicry. *Behaviour* **24**(3): 269–282. doi:10.1163/156853965X00066.
- Dyer, A.G., and Neumeyer, C. 2005. Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **191**(6): 547–557. doi:10.1007/s00359-005-0622-z.
- Edmunds, M. 2000. Why are there good and poor mimics? *Biol. J. Linn. Soc.* **70**(3): 459–466. doi:10.1006/bjil.1999.0425.
- Edwards, P.J. 1989. Insect herbivory and plant defence theory. *In* *Towards a More Exact Ecology*. Edited by P.J. Grubb and J.B. Whittaker. Blackwell Scientific Publications, Oxford, UK. pp. 275–297.
- Ehleringer, J.R., Ullmann, I., Lange, O.L., Farquhar, G.D., Cowan, I.R., Schulze, E.D., and Ziegler, H. 1986. Mistletoes: a hypothesis concerning morphological and chemical avoidance of herbivory. *Oecologia* **70**(2): 234–237. doi:10.1007/BF00379245.
- Ehrlich, P.R., and Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**(4): 586–608. doi:10.1111/j.1558-5646.1964.tb01674.x.

- Endara, M.J., and Coley, P.D. 2011. The resource availability hypothesis revisited: a meta-analysis. *Funct. Ecol.* **25**(2): 389–398. doi:10.1111/j.1365-2435.2010.01803.x.
- Endler, J.A. 1981. An overview of the relationship between mimicry and crypsis. *Biol. J. Linnaean Soc.* **16**(1): 25–31. doi:10.1111/j.1095-8312.1981.tb01840.x.
- Endler, J.A. 1991. Interactions between predators and prey. *In Behavioural Ecology. An Evolutionary Approach*. Edited by J.R. Krebs and N.B. Davies. Blackwell Science Ltd, Cambridge, UK. pp. 169–196.
- Endler, J.A., and Mappes, J. 2004. Predator mixes and the conspicuousness of aposematic signals. *Am. Nat.* **163**(4): 532–47. doi:10.1086/382662.
- Elliott, S., and Loudon, A. 1987. Effects of monoterpene odors on food selection by red deer calves (*Cervus elaphus*). *J. Chem. Ecol.* **13**(6): 1343–1349. doi:10.1007/BF01012281.
- Fadzly, N., and Burns, K.C. 2010. Hiding from the ghost of herbivory past: evidence for crypsis in an insular tree species. *Int. J. Plant Sci.* **171**(8): 828–833. doi:10.1086/654850.
- Fadzly, N., Jack, C., Schaefer, H.M., and Burns, K.C. 2009. Ontogenetic colour changes in an insular tree species: signalling to extinct browsing birds? *New Phytol.* **184**(2): 495–501. doi:10.1111/j.1469-8137.2009.02926.x.
- Feeny, P. 1970. Seasonal changes in oak leaf Tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**(4): 565–581. doi:10.2307/1934037.
- Ford, E.B. 1953. The genetics of polymorphism in the Lepidoptera. *Adv. Genet.* **5**: 43–87. doi:10.1016/S0065-2660(08)60405-8.
- Forsyth, D.M., Coomes, D.A., and Nugent, G. 2003. Framework for assessing the susceptibility of management areas to deer impacts. In *Science for Conservation*. Wellington. Available from <http://conservation.govt.nz/Documents/science-and-technical/sfc213.pdf>.
- Forsyth, D.M., Coomes, D.A., Nugent, G., and Hall, G.M.J. 2002. Diet and diet preferences of introduced ungulates (Order: Artiodactyla) in New Zealand. *New Zeal. J. Zool.* **29**(4): 323–343. doi:10.1080/03014223.2002.9518316.

- Forsyth, D.M., Richardson, S.J., and Menchenton, K. 2005. Foliar fibre predicts diet selection by invasive Red Deer *Cervus elaphus scoticus* in a temperate New Zealand forest. *Funct. Ecol.* **19**(3): 495–504. doi:10.1111/j.1365-2435.2005.00991.x.
- Forsyth, D.M., Wilmschurst, J.M., Allen, R.B., and Coomes, D.A. 2010. Impacts of introduced deer and extinct moa on New Zealand ecosystems. *N. Z. J. Ecol.* **34**(1): 48–65.
- Franks, D.W., Ruxton, G.D., and Sherratt, T.N. 2009. Warning signals evolve to disengage Batesian mimics. *Evolution* **63**(1): 256–267. doi:10.1111/j.1558-5646.2008.00509.x.
- Fraser, K.W., Cone, J.M., and Whitford, E.J. 2000. A revision of the established ranges and new populations of 11 introduced ungulate species in New Zealand. *J. R. Soc. New Zeal.* **30**(4): 419–437. doi:10.1080/03014223.2000.9517633.
- Fraser, S., Callahan, A., Klassen, D., and Sherratt, T.N. 2007. Empirical tests of the role of disruptive coloration in reducing detectability. *Proc. R. Soc. B Biol. Sci.* **274**(1615): 1325–1331. doi:10.1098/rspb.2007.0153.
- Freeland, W.J., and Janzen, D.H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* **108**(961): 269–289. doi:10.1086/282907.
- Gamberale-Stille, G., and Tullberg, B.S. 1999. Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey. *Evol. Ecol.* **13**(6): 579–589. doi:10.1023/A:1006741626575.
- Gaskett, A. 2011. Orchid pollination by sexual deception: pollinator perspectives. *Biol. Rev. Camb. Philos. Soc.* **86**(1): 33–75. doi:10.1111/j.1469-185X.2010.00134.x.
- Getty, T. 1985. Discriminability and the sigmoid functional response: how optimal foragers could stabilize model-mimic complexes. *Am. Nat.* **125**(2): 239–256. doi:10.1086/284339.
- Gianoli, E., and Carrasco-Urra, F. 2014. Leaf mimicry in a climbing plant protects against herbivory. *Curr. Biol.* **24**(9): 984–987. doi:10.1016/j.cub.2014.03.010.
- Gittleman, J.L., and Harvey, P.H. 1980. Why are distasteful prey not cryptic? *Nature* **286**(5769): 149–150. doi:10.1038/286149a0.

- Giurfa, M., and Menzel, R. 1997. Insect visual perception: complex abilities of simple nervous systems. *Curr. Opin. Neurobiol.* **7**(4): 505–13. doi:10.1016/S0959-4388(97)80030-X.
- Giurfa, M., Vorobyev, M., Kevan, P., and Menzel, R. 1996. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J. Comp. Physiol. A* **178**(5): 699–709. doi:10.1007/BF00227381.
- Gould, K.S. 2004. Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *J. Biomed. Biotechnol.* **2004**(5): 314–320. doi:10.1155/S1110724304406147.
- Gould, K.S. 2010. Muriel Wheldale Onslow and the rediscovery of anthocyanin function in plants. *In* Recent Advances in Polyphenol Research, Volume 2. Edited by C. Santos-Buelga, M.T. Escribano-Bailon, and V. Lattanzio. Wiley-Blackwell, Oxford, UK. pp. 206–225. doi:0.1002/9781444323375.ch7.
- Gould, K.S., Dudle, D.A., and Neufeld, H.S. 2010. Why some stems are red: cauline anthocyanins shield photosystem II against high light stress. *J. Exp. Bot.* **61**(10): 2707–2717. doi:10.1093/jxb/erq106.
- Gould, K.S., Markham, K.R., Smith, R.H., and Goris, J.J. 2000. Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Cunn. *J. Exp. Bot.* **51**(347): 1107–1115. doi:10.1093/jexbot/51.347.1107.
- Green, J.P., Foster, R., Wilkins, L., Osorio, D., and Hartley, S.E. 2015. Leaf colour as a signal of chemical defence to insect herbivores in wild cabbage (*Brassica oleracea*). *PLoS One* **10**(9): 1–20. doi:10.1371/journal.pone.0136884.
- Greene, H.W., and McDiarmid, R.W. 1981. Coral snake mimicry: does it occur? *Science* **213**(4513): 1207–1212. doi:10.1126/science.213.4513.1207.
- Greenwood, R.M., and Atkinson, I.A.E. 1977. Evolution of divaricating plants in New Zealand in relation to moa browsing. *Proc. New Zeal. Ecol. Soc.* **24**: 21–33. Available from <http://www.jstor.org/stable/24052033> [accessed 11 February 2013].
- Griffiths, C.J., Jones, C.G., Hansen, D.M., Puttoo, M., Tatayah, R. V., Müller, C.B., and Harris, S. 2010. The use of extant non-indigenous tortoises as a restoration tool to replace extinct ecosystem engineers. *Restor. Ecol.* **18**(1): 1–7. doi:10.1111/j.1526-100X.2009.00612.x.

- Guillet, G., Philogène, B.J.R., O'Meara, J., Durst, T., and Arnason, J.T. 1997. Multiple modes of insecticidal action of three classes of polyacetylene derivatives from *Rudbeckia hirta*. *Phytochemistry* **46**(3): 495–498. doi:10.1016/S0031-9422(97)00320-8.
- Gurevitch, J., and Schuepp, P.H. 1990. Boundary layer properties of highly dissected leaves: an investigation using an electrochemical fluid tunnel. *Plant. Cell Environ.* **13**(8): 783–792. doi:10.1111/j.1365-3040.1990.tb01094.x.
- Halpin, C.G., Skelhorn, J., and Rowe, C. 2008. Being conspicuous and defended: selective benefits for the individual. *Behav. Ecol.* **19**(5): 1012–1017. doi:10.1093/beheco/arn069.
- Halpin, C.G., Skelhorn, J., and Rowe, C. 2014. Increased predation of nutrient-enriched aposematic prey. *Proc. R. Soc. B Biol. Sci.* **281**(1781): 20133255–20133255. doi:10.1098/rspb.2013.3255.
- Halpin, C.G., Skelhorn, J., Rowe, C., Ruxton, G.D., and Higginson, A.D. 2017. The impact of detoxification costs and predation risk on foraging: implications for mimicry dynamics. *PLoS One* **12**(1): 1–18. doi:10.1371/journal.pone.0169043.
- Ham, A.D., Ihalaenen, E., Lindström, L., and Mappes, J. 2006. Does colour matter? The importance of colour in avoidance learning, memorability and generalisation. *Behav. Ecol. Sociobiol.* **60**(4): 482–491. doi:10.1007/s00265-006-0190-4.
- Hamilton, W.D., and Brown, S.P. 2001. Autumn tree colours as a handicap signal. *Proc. R. Soc. B Biol. Sci.* **268**(1475): 1489–1493. doi:10.1098/rspb.2001.1672.
- Hammer, Ø., Harper, D.A.T., and Ryan, P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **4**(1): 1–9. doi:10.1016/j.bcp.2008.05.025.
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M., and Rafferty, C.M. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol. Evol. Syst.* **8**(4): 157–178. doi:10.1016/j.ppees.2007.01.001.
- Harper, G.R., and Pfennig, D.W. 2007. Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? *Proc. R. Soc. B Biol. Sci.* **274**(1621): 1955–1961. doi:10.1098/rspb.2007.0558.

- Harper, G.R., and Pfennig, D.W. 2008. Selection overrides gene flow to break down maladaptive mimicry. *Nature* **451**(7182): 1103–1106. doi:10.1038/nature06532.
- Hartley, S.E., and Jones, C.G. 1986. Plant chemistry and herbivory, or why the world is green. *In* *Plant Ecology*, 2nd edition. Edited by M.J. Crawley. Blackwell Science Ltd, Oxford, UK. pp. 284–324. doi:10.1002/9781444313642.ch10.
- Harvey, P.H. 1983. Why some insects look pretty nasty. *New Sci.* **97**(1339): 26–27.
- Hasson, O. 1991. Sexual displays as amplifiers: practical examples with an emphasis on feather decorations. *Behav. Ecol.* **2**(3): 189–197. doi:10.1093/beheco/2.3.189.
- Hawkes, C. V., and Sullivan, J.J. 2001. The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology* **82**(7): 2045–2058. doi:10.1890/0012-9658(2001)082[2045:TIOHOP]2.0.CO;2.
- Hazel, W.N. 2002. The environmental and genetic control of seasonal polyphenism in larval color and its adaptive significance in a swallowtail butterfly. *Evolution*. **56**(2): 342–348. doi:10.1111/j.0014-3820.2002.tb01344.x.
- Hebets, E.A. 2005. Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behav. Ecol.* **16**(1): 75–82. doi:10.1093/beheco/arh133.
- Hebets, E.A., and Papaj, D.R. 2005. Complex signal function: Developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* **57**(3): 197–214. doi:10.1007/s00265-004-0865-7.
- Hebets, E.A., and Uetz, G.W. 1999. Females responses to isolated signals from multimodal male courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Anim. Behav.* **57**(4): 865–872. doi:10.1006/anbe.1998.1048.
- Hegazy, A.K., and El Amry, M.I. 1998. Leaf temperature of desert sand dune plants: Perspectives on the adaptability of leaf morphology. *Afr. J. Ecol.* **36**(1): 34–43. doi:10.1046/j.1365-2028.1998.109- 89109.x.
- Hernández, H.M., Gómez-Hinostrosa, C., and Bárcenas, R.T. 2001. Diversity, spatial arrangement, and endemism of Cactaceae in the Huizache area, a hot-spot in the Chihuahuan Desert. *Biodivers. Conserv.* **10**(7): 1097–1112. doi:10.1023/A:101660621

- Hetz, M., and Slobodchikoff, C. 1988. Predation pressure on an imperfect Batesian mimicry complex in the presence of alternative prey. *Oecologia* **76**(4): 570–573. doi:10.1007/BF00397872.
- Holen, Ø.H. 2013. Disentangling taste and toxicity in aposematic prey. *Proc. R. Soc. B Biol. Sci.* **280**(1753): 20122588. doi:10.1098/rspb.2012.2588.
- Holling, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. *Mem. Entomol. Soc. Canada* **97**(45): 1–60. doi:10.4039/entm9745fv.
- Horn, H.S. 1971. The adaptive geometry of trees. Princeton University Press, Princeton.
- Hovenden, M.J., and Vander Schoor, J.K. 2006. The response of leaf morphology to irradiance depends on altitude of origin in *Nothofagus cunninghamii*. *New Phytol.* **169**(2): 291–297. doi:10.1111/j.1469-8137.2005.01585.x.
- Howse, P.E., and Allen, J.A. 1994. Satyric mimicry: the evolution of apparent imperfection. *Proc. R. Soc. B Biol. Sci.* **257**(1349): 111–114. doi:10.1098/rspb.1994.0102.
- Hughes, N.M. 2011. Winter leaf reddening in “evergreen” species. *New Phytol.* **190**(3): 573–581. doi:10.1111/j.1469-8137.2011.03662.x.
- Hughes, N.M., and Lev-Yadun, S. 2015. Red/purple leaf margin coloration: potential ecological and physiological functions. *Environ. Experimental Bot.* **119**: 27–39. doi:10.1016/j.envexpbot.2015.05.015.
- Hughes, N.M., Neufeld, H.S., and Burkey, K.O. 2005. Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. *New Phytol.* **168**(3): 575–587. doi:10.1111/j.1469-8137.2005.01546.x.
- Husheer, S.W., Coomes, D.A., and Robertson, A.W. 2003. Long-term influences of introduced deer on the composition and structure of New Zealand *Nothofagus* forests. *For. Ecol. Manage.* **181**(1–2): 99–117. doi:10.1016/S0378-1127(03)00120-8.
- Huxley, J.S. 1966. A discussion on ritualization of behaviour in animals and man – Introduction. *Philos. Trans. R. Soc. B Biol. Sci.* **251**(772): 247–271. doi:10.1098/rstb.1966.0010.

- Ihalainen, E., Lindström, L., Mappes, J., and Puolakkainen, S. 2007. Can experienced birds select for Müllerian mimicry? *Behav. Ecol.* **19**(2): 362–368.
doi:10.1093/beheco/arm151.
- Ihalainen, E., Rowland, H.M., Speed, M.P., Ruxton, G.D., and Mappes, J. 2012. Prey community structure affects how predators select for Müllerian mimicry. *Proc. R. Soc. B Biol. Sci.* **279**(1736): 2099–2105. doi:10.1098/rspb.2011.2360.
- Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., Van Ruijven, J., Weigelt, A., Wilsey, B.J., Zavaleta, E.S., and Loreau, M. 2011. High plant diversity is needed to maintain ecosystem services. *Nature* **477**(7363): 199–202. doi:10.1038/nature10282.
- Iserbyt, A., Bots, J., Dongen, S. Van, Ting, J.J., Gossum, H. Van, Sherratt, T.N., Marsh, B., and Marsh, O. 2011. Frequency-dependent variation in mimetic fidelity in an intraspecific mimicry system. *Proc. R. Soc. B Biol. Sci.* **278**(1721): 3116–3122.
doi:10.1098/rspb.2011.0126.
- Jermy, T. 1976. Insect—host-plant relationship — co-evolution or sequential evolution? *In* The Host-Plant in Relation to Insect Behaviour and Reproduction. Edited by T. Jermy. Springer, Boston, MA. pp. 109–113. doi:doi.org/10.1007/978-1-4613-4274-8.
- Johnstone, R.A. 2002. The evolution of inaccurate mimics. *Nature* **418**(6897): 524–526.
doi:10.1038/nature00845.
- Jones, C.G., Lawton, J.H., and Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos* **69**(3): 373–386. doi:10.2307/3545850.
- Jones, D.A. 1962. Selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. *Nature* **193**(4820): 1109–1110. doi:10.1038/1931109a0.
- Joron, M. 2008. Batesian mimicry: can a leopard change its spots — and get them back? *Curr. Biol.* **18**(11): R476–479. doi:10.1016/j.cub.2008.04.031.
- Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* **25**(2): 339–347. doi:10.1111/j.1365-2435.2010.01789.x.
- Karban, R., and Agrawal, A.A. 2002. Herbivore offense. *Annu. Rev. Ecol. Syst.* **33**: 641–664.
doi:10.1146/annurev.ecolsys.33.010802.150443.

- Karban, R., Agrawal, A.A., Thaler, J.S., and Adler, L.S. 1999. Induced plant responses and information content about risk of herbivory. *Trends Ecol. Evol.* **14**(11): 443–447. doi:10.1016/S0169-5347(99)01678-X.
- Karban, R., and Myers, J.H. 1989. Induced plant responses to herbivory. *Annu. Rev. Ecol. Syst.* **20**: 331–348. doi:10.1146/annurev.es.20.110189.001555.
- Kazemi, B., Gamberale-Stille, G., and Leimar, O. 2015. Multi-trait mimicry and the relative salience of individual traits. *Proc. R. Soc. B Biol. Sci.* **282**(1818): 20152127. doi:10.1098/rspb.2015.2127.
- Kazemi, B., Gamberale-Stille, G., Tullberg, B.S., and Leimar, O. 2014. Stimulus salience as an explanation for imperfect mimicry. *Curr. Biol.* **24**(9): 965–969. doi:10.1016/j.cub.2014.02.061.
- Kelman, E., Baddeley, R., Shohet, A., and Osorio, D. 2007. Perception of visual texture and the expression of disruptive camouflage by the cuttlefish, *Sepia officinalis*. *Proc. R. Soc. B Biol. Sci.* **274**(1616): 1369–1375. doi:10.1098/rspb.2007.0240.
- Kidner, C.A., and Umbreen, S. 2010. Why is leaf shape so variable? *Int. J. Plant Dev. Biol.* **4**(1): 64–75.
- Kikuchi, D.W., Malick, G., Webster, R.J., Whissell, E., and Sherratt, T.N. 2015. An empirical test of 2-dimensional signal detection theory applied to Batesian mimicry. *Behav. Ecol.* **26**(4): 1226–1235. doi:10.1093/beheco/arv072.
- Kikuchi, D.W., Mappes, J., Sherratt, T.N., and Valkonen, J.K. 2016. Selection for multicomponent mimicry: equal feature salience and variation in preferred traits. *Behav. Ecol.* **27**(5): 1515–1521. doi:10.1093/beheco/arw072.
- Kikuchi, D.W., and Pfennig, D.W. 2010a. High-model abundance may permit the gradual evolution of Batesian mimicry: an experimental test. *Proc. R. Soc. B Biol. Sci.* **277**(1684): 1041–8. doi:10.1098/rspb.2009.2000.
- Kikuchi, D.W., and Pfennig, D.W. 2010b. Predator cognition permits imperfect coral snake mimicry. *Am. Nat.* **176**(6): 830–834. doi:10.1086/657041.
- Kikuchi, D.W., and Pfennig, D.W. 2012. A Batesian mimic and its model share color production mechanisms. *Curr. Zool.* **58**(4): 658–667. doi:10.1093/czoolo/58.4.658.

- Kikuchi, D.W., and Pfennig, D.W. 2013. Imperfect mimicry and the limits of natural selection. *Q. Rev. Biol.* **88**(4): 297–315. doi:10.1086/673758.
- King, C.M. 1990. *The Handbook of New Zealand Mammals*. Oxford University Press, Auckland.
- Klingenberg, C.P. 2010. Evolution and development of shape: integrating quantitative approaches. *Nat. Rev. Genet.* **11**(9): 623–35. doi:10.1038/nrg2829.
- Klingenberg, C.P., Duttke, S., Whelan, S., and Kim, M. 2012. Developmental plasticity, morphological variation and evolvability: a multilevel analysis of morphometric integration in the shape of compound leaves. *J. Evol. Biol.* **25**(1): 115–29. doi:10.1111/j.1420-9101.2011.02410.x.
- Klooster, M.R., Clark, D.L., and Culley, T.M. 2009. Cryptic bracts facilitate herbivore avoidance in the mycoheterotrophic plant *Monotropsis odorata* (Ericaceae). *Am. J. Bot.* **96**(12): 2197–2205. doi:10.3732/ajb.0900124.
- Koricheva, J., Nykänen, H., and Gianoli, E. 2004. Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *Am. Nat.* **163**(4): E64–E75. doi:10.1086/382601.
- Kotanen, P.M., and Rosenthal, J.P. 2000. Tolerating herbivory: does the plant care if the herbivore has a backbone? *Evol. Ecol.* **17**(4–6): 537–549. doi:10.1023/A:1010862201331.
- Koziel, J., Jia, M., Khaled, A., Noah, J., and Pawliszyn, J. 1999. Field air analysis with SPME device. *Anal. Chim. Acta* **400**(1–3): 153–162. doi:10.1016/S0003-2670(99)00614-5.
- Kraemer, A.C., Serb, J.M., and Adams, D.C. 2015. Batesian mimics influence the evolution of conspicuousness in an aposematic salamander. *J. Evol. Biol.* **28**(5): 1016–1023. doi:10.1111/jeb.12622.
- Lankau, R.A. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytol.* **175**(1): 176–184. doi:10.1111/j.1469-8137.2007.02090.x.

- Launchbaugh, K.L., Provenza, F.D., and Burritt, E.A. 1993. How herbivores track variable environments: response to variability of phytotoxins. *J. Chem. Ecol.* **19**(6): 1047–1056. doi:10.1007/BF00987367.
- Launchbaugh, K.L.K.K.L., and Provenza, F.D. 1993. Can plants practice mimicry to avoid grazing by mammalian herbivores? *Oikos* **66**(3): 501–504. doi:10.2307/3544945.
- Lee, D.W. 2002. Anthocyanins in leaves: distribution, phylogeny and development. *Adv. Bot. Res. Acad. Press* **37**: 37–53. doi:10.1016/S0065-2296(02)37042-3.
- Lee, D.W. 2007. *Nature's palette*. The University of Chicago Press, Chicago and London.
- Lee, W.G., Wood, J.R., and Rogers, G.M. 2010. Legacy of avian-dominated plant-herbivore systems in New Zealand. *N. Z. J. Ecol.* **34**(1): 28–47.
- Leigh, A., Zwieniecki, M.A., Rockwell, F.E., Boyce, C.K., Nicotra, A.B., and Holbrook, N.M. 2011. Structural and hydraulic correlates of heterophylly in *Ginkgo biloba*. *New Phytol.* **189**(2): 459–470. doi:10.1111/j.1469-8137.2010.03476.x.
- Leonard, A.S.A., Dornhaus, A., and Papaj, D.R.D.R. 2011. Flowers help bees cope with uncertainty: signal detection and the function of floral complexity. *J. Exp. Biol.* **214**(1): 113–121. doi:10.1242/jeb.047407.
- Lev-Yadun, S. 2001. Aposematic (warning) coloration associated with thorns in higher plants. *J. Theor. Biol.* **210**(3): 385–388. doi:10.1006/jtbi.2001.2315.
- Lev-Yadun, S. 2003. Weapon (thorn) automimicry and mimicry of aposematic colorful thorns in plants. *J. Theor. Biol.* **224**(2): 183–188. doi:10.1016/S0022-5193(03)00156-5.
- Lev-Yadun, S. 2016. *Defensive (anti-herbivory) Coloration in Land Plants*. Springer, Cham. doi:10.1007/978-3-319-42096-7.
- Lev-Yadun, S., Dafni, A., Flaishman, M.A., Inbar, M., Izhaki, I., Katzir, G., and Ne'eman, G. 2004. Plant coloration undermines herbivorous insect camouflage. *BioEssays* **26**(10): 1126–1130. doi:10.1002/bies.20112.
- Lindstedt, C., Eager, H., Ihalainen, E., Kahilainen, A., Stevens, M., and Mappes, J. 2011. Direction and strength of selection by predators for the color of the aposematic wood tiger moth. *Behav. Ecol.* **22**(3): 580–587. doi:10.1093/beheco/arr017.

- Lindstedt, C., Lindström, L., and Mappes, J. 2008a. Thermoregulation constrains effective warning signal expression. *Evolution* **63**(2): 469–478. doi:10.1111/j.1558-5646.2008.00561.x.
- Lindstedt, C., Lindström, L., and Mappes, J. 2008b. Hairiness and warning colours as components of antipredator defence: additive or interactive benefits. *Anim. Behav.* **75**(5): 1703–1713. doi:dx.doi.org/10.1016/j.anbehav.2007.10.024.
- Lindstedt, C., Lindström, L., and Mappes, J. 2009. Thermoregulation constrains effective warning signal expression. *Evolution* **63**(2): 469–478. doi:10.1111/j.1558-5646.2008.00561.x.
- Lindström, L., Alatalo, R. V., Lyytinen, A., and Mappes, J. 2001a. Predator experience on cryptic prey affects the survival of conspicuous aposematic prey. *Proc. R. Soc. B Biol. Sci.* **268**(1465): 357–361. doi:10.1098/rspb.2000.1377.
- Lindström, L., Alatalo, R. V., Lyytinen, A., and Mappes, J. 2001b. Strong antiapostatic selection against novel rare aposematic prey. *Proc. Natl. Acad. Sci. U. S. A.* **98**(16): 9181–9184. doi:10.1073/pnas.161071598.
- Lindström, L., Alatalo, R. V., Lyytinen, A., and Mappes, J. 2004. The effect of alternative prey on the dynamics of imperfect Batesian and Müllerian mimics. *Evolution* **58**(6): 1294–1302. doi:10.1554/03-271.
- Lindström, L., Alatalo, R. V., and Mappes, J. 1997. Imperfect Batesian mimicry - the effects of the frequency and the distastefulness of the model. *Proc. R. Soc. B Biol. Sci.* **264**(1379): 149–153. doi:10.1098/rspb.1997.0022.
- Lindström, L., Alatalo, R. V., Mappes, J., Riipi, M., and Vertanen, L. 1999. Can aposematic signals evolve by gradual change? *Nature* **397**(6716): 249–251. doi:10.1038/16692.
- Lorenz, K.Z. 1966. Evolution of ritualization in the biological and cultural spheres. *Philos. Trans. R. Soc. B Biol. Sci.* **251**(772): 273–284. doi:10.1098/rstb.1966.0011.
- Lynn, S.K., Cnaani, J., and Papaj, D.R. 2005. Peak shift discrimination learning as a mechanism of social evolution. *Evolution* **59**(6): 1300–1305. doi:10.1111/j.0014-3820.2005.tb01780.x.

- Macaluso, E., Frith, C.D., and Driver, J. 2000. Modulation of human visual cortex by crossmodal spatial attention. *Science* **289**(5482): 1206–1208.
doi:10.1126/science.289.5482.1206.
- Macel, M., and Vrieling, K. 2003. Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, *Tyria jacobaeae*. *J. Chem. Ecol.* **29**(6): 1435–1446.
doi:10.1023/A:1024269621284.
- Mackay, D.A., and Jones, R.E. 1989. Leaf shape and the host-finding behaviour of two ovipositing monophagous butterfly species. *Ecol. Entomol.* **14**(4): 423–431.
doi:10.1111/j.1365-2311.1989.tb00944.x.
- Mackintosh, N.J. 1976. Overshadowing and stimulus intensity. *Anim. Learn. Behav.* **4**(2): 186–192. doi:doi.org/10.3758/BF03214033.
- Mappes, J., and Alatalo, R. V. 1997. Effects of novelty and gregariousness in survival of aposematic prey. *Behav. Ecol.* **8**(2): 174–177. doi:10.1093/beheco/8.2.174.
- Maron, J.L., and Crone, E. 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proc. R. Soc. B Biol. Sci.* **273**(1601): 2575–2584.
doi:10.1098/rspb.2006.3587.
- Marquis, R.J. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science* **226**(4674): 537–539. doi:10.1126/science.226.4674.537.
- Marsh, K.J., Wallis, I.R., and Foley, W.J. 2005. Detoxification rates constrain feeding in common brushtail possums (*Trichosurus vulpecula*). *Ecology* **86**(11): 2946–2954.
doi:10.1890/05-0303.
- Marsh, K.J., Wallis, I.R., and Foley, W.J. 2007. Behavioural contributions to the regulated intake of plant secondary metabolites in koalas. *Oecologia* **154**(2): 283–290.
doi:10.1007/s00442-007-0828-6.
- Marsh, K.J., Wallis, I.R., McLean, S., Sorensen, J.S., and Foley, W.J. 2006. Conflicting demands on detoxification pathways influence how common brushtail possums choose their diets. *Ecology* **87**(8): 2103–2112. doi:10.1890/0012-9658(2006)87[2103:CDODPI]2.0.CO;2.
- Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., and Wolpert, L. 1985. Developmental constraints and evolution: a

- perspective from the mountain lake conference on development and evolution. *Q. Rev. Biol.* **60**(3): 265–287. doi:10.1086/414425.
- Maynard Smith, J., and Harper, D.G.C. 2003. *Animal signals*. Oxford University Press, Oxford, UK.
- McDonald, P.G., Fonseca, C.R., Overton, J.M., and Westoby, M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Funct. Ecol.* **17**(1): 50–57. doi:10.1046/j.1365-2435.2003.00698.x.
- McGlone, M.S., and Webb, C.J. 1981. Selective forces influencing the evolution of divaricating plants. *N. Z. J. Ecol.* **4**(1963): 20–28. Available from <http://www.jstor.org/stable/24052601>.
- McGuire, L., Van Gossum, H., Beirinckx, K., and Sherratt, T.N. 2006. An empirical test of signal detection theory as it applies to Batesian mimicry. *Behav. Processes* **73**(3): 299–307. doi:10.1016/j.beproc.2006.07.004.
- McKey, D. 1974. Adaptive patterns in alkaloid physiology. *Am. Nat.* **108**(961): 305–320. doi:10.1086/282909.
- McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* **40**(3): 329–336. doi:10.2307/3544305.
- Merilaita, S., and Kaitala, V. 2002. Community structure and the evolution of aposematic coloration. *Ecol. Lett.* **5**(4): 495–501. doi:10.1046/j.1461-0248.2002.00362.x.
- Merilaita, S., and Ruxton, G.D. 2007. Aposematic signals and the relationship between conspicuousness and distinctiveness. *J. Theor. Biol.* **245**(2): 268–277. doi:10.1016/j.jtbi.2006.10.022.
- Milla, R., and Reich, P.B. 2011. Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. *Ann. Bot.* **107**(3): 455–65. doi:10.1093/aob/mcq261.
- Mitchell, R.J., Fordham, R.A., and John, A. 1987. The annual diet of feral goats (*Capra hircus* L.) in lowland rimu-rata-kamahi forest on eastern Mount Taranaki (Mt Egmont). *New Zeal. J. Zool.* **14**(2): 179–192. doi:10.1080/03014223.1987.10422988.

- Müller, F. 1879. *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. Trans. Entomol. Soc. London: xx–xxix. doi:10.1017/CBO9781107415324.004.
- Neill, S.O., and Gould, K.S. 2003. Anthocyanins in leaves: light attenuators or antioxidants? Funct. Plant Biol. **30**(8): 865–873.
- Nicotra, A.B., Leigh, A., Boyce, C.K., Jones, C.S., Niklas, K.J., Royer, D.L., and Tsukaya, H. 2011. The evolution and functional significance of leaf shape in the angiosperms. Funct. Plant Biol. **38**(7): 535–552. doi:10.1071/FP11057.
- Niemelä, P., Tuomi, J. 1987. Does the leaf morphology of some plants mimic caterpillar damage? Oikos **50**(2): 256–257. doi:10.2307/3566009.
- Nieminen, M., Suomi, J., Van Nouhuys, S., Sauri, P., and Riekkola, M.L. 2003. Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. J. Chem. Ecol. **29**(4): 823–844. doi:10.1023/A:1022923514534.
- Nishio, J.N. 2000. Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. Plant, Cell Environ. **23**(6): 539–548. doi:10.1046/j.1365-3040.2000.00563.x.
- Niskanen, M., and Mappes, J. 2005. Significance of the dorsal zigzag pattern of *Vipera latastei gaditana* against avian predators. J. Anim. Ecol. **74**(6): 1091–1101. doi:10.1111/j.1365-2656.2005.01008.x.
- Nugent, G., Fraser, K.W., Asher, G.W., and Tustin, K.G. 2001. Advances in New Zealand mammalogy 1990–2000: Deer. J. R. Soc. New Zeal. **31**(1): 263–298. doi:10.1080/03014223.2001.9517654.
- Nugent, G., Fraser, K.W., and Sweetapple, P.J. 1997. Comparison of red deer and possum diets and impacts podocarp-hardwood forest, Waihaha Catchment, Pureora Conservation Park. In Science for Conservation. Wellington. Available from <http://nationalparks.co.nz/Documents/science-and-technical/Sfc050.pdf>.
- Nur, U. 1970. Evolutionary rates of models and mimics in Batesian mimicry. Am. Nat. **104**(939): 477–486. doi:10.1086/282682.
- Ojala, K., Lindström, L., and Mappes, J. 2007. Life-history constraints and warning signal expression in an arctiid moth. Funct. Ecol. **21**(6): 1162–1167. doi:10.1111/j.1365-2435.2007.01322.x.

- Otte, D. 1974. Effects and functions in the evolution of signaling systems. *Annu. Rev. Ecol. Syst.* **5**: 385–417. doi:10.1146/annurev.es.05.110174.002125.
- Ougham, H.J., Morris, P., and Thomas, H. 2005. The colors of autumn leaves as symptoms of cellular recycling and defenses against environmental stresses. *Curr. Top. Dev. Biol.* **66**: 135–160. doi:10.1016/S0070-2153(05)66004-8.
- Paige, K.N., and Whitham, T.G. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am. Nat.* **129**(3): 407–416. doi:10.1086/284645.
- Parkes, J.P. 1993. Feral goats: designing solutions for a designer pest. *N. Z. J. Ecol.* **17**(2): 71–83.
- Parsons, M., Koch, J., Lamont, B., Vlahos, S., and Fairbanks, M. 2006. Planting density effects and selective herbivory by kangaroos on species used in restoring forest communities. *For. Ecol. Manage.* **229**(1–3): 39–49. doi:10.1016/j.foreco.2006.03.020.
- Pekár, S., Jarab, M., Fromhage, L., and Herberstein, M.E. 2011. Is the evolution of inaccurate mimicry a result of selection by a suite of predators? A case study using myrmecomorphic spiders. *Am. Nat.* **178**(1): 124–134. doi:10.1086/660287.
- Penney, H.D., Hassall, C., Skevington, J.H., Abbott, K.R., and Sherratt, T.N. 2012. A comparative analysis of the evolution of imperfect mimicry. *Nature* **483**(7390): 461–464. doi:10.1038/nature10961.
- Peppe, D.J., Royer, D.L., Cariglino, B., Oliver, S.Y., Newman, S., Leight, E., Enikolopov, G., Fernandez-Burgos, M., Herrera, F., Adams, J.M., Correa, E., Currano, E.D., Erickson, J.M., Hinojosa, L.F., Hoganson, J.W., Iglesias, A., Jaramillo, C. a, Johnson, K.R., Jordan, G.J., Kraft, N.J.B., Lovelock, E.C., Lusk, C.H., Niinemets, U., Peñuelas, J., Rapson, G., Wing, S.L., and Wright, I.J. 2011. Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. *New Phytol.* **190**(3): 724–39. doi:10.1111/j.1469-8137.2010.03615.x.
- Perry, G.L.W., Wheeler, A.B., Wood, J.R., and Wilmshurst, J.M. 2014. A high-precision chronology for the rapid extinction of New Zealand moa (Aves, Dinornithiformes). *Quat. Sci. Rev.* **105**: 126–135. doi:10.1016/j.quascirev.2014.09.025.

- Pfennig, D.W., Harcombe, W.R., and Pfennig, K.S. 2001. Frequency-dependent Batesian mimicry. *Nature* **410**(6826): 323. doi:10.1038/35066628.
- Pfennig, D.W., Harper, G.R., Brumo, A.F., Harcombe, W.R., and Pfennig, K.S. 2007. Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. *Behav. Ecol. Sociobiol.* **61**(4): 505–511. doi:10.1007/s00265-006-0278-x.
- Pfennig, D.W., and Kikuchi, D.W. 2012. Competition and the evolution of imperfect mimicry. *Curr. Zool.* **58**(4): 608–619. doi:10.1093/czoolo/58.4.608.
- Pfennig, D.W., and Mullen, S.P. 2010. Mimics without models: causes and consequences of allopatry in Batesian mimicry complexes. *Proc. R. Soc. B Biol. Sci.* **277**(1694): 2577–2585. doi:10.1098/rspb.2010.0586.
- Poisot, T., Kéfi, S., Morand, S., Stanko, M., Marquet, P.A., and Hochberg, M.E. 2015. A continuum of specialists and generalists in empirical communities. *PLoS One* **10**(5): 1–12. doi:10.1371/journal.pone.0114674.
- Pollock, M.L., Lee, W.G., Walker, S., and Forrester, G. 2007. Ratite and ungulate preferences for woody New Zealand plants: Influence of chemical and physical traits. *N. Z. J. Ecol.* **31**(1): 68–78.
- Poulton, E.B. 1890. The colours of animals: their meaning and use especially considered in the case of insects. Keegan Paul, Trench & Trübner, London.
- Provenza, F.D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *J. Range Manag.* **48**(1): 2–17. doi:10.2307/4002498.
- Prudic, K.L., Skemp, A.K., and Papaj, D.R. 2007. Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behav. Ecol.* **18**(1): 41–46. doi:10.1093/beheco/arl046.
- Pulliam, H.R. 1974. On the theory of optimal diets. *Am. Nat.* **108**(959): 59–74. doi:10.1086/282885.
- Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U., and Vilà, M. 2012. A global assessment of invasive plant impacts on resident species, communities and ecosystems: The interaction of impact measures, invading species' traits and

- environment. *Glob. Chang. Biol.* **18**(5): 1725–1737. doi:10.1111/j.1365-2486.2011.02636.x.
- Raubenheimer, D., Mayntz, D., Simpson, S.J., and Tøft, S. 2007. Nutrient-specific compensation following diapause in a predator: implications for intraguild predation. *Ecology* **88**(10): 2598–2608. doi:10.1890/07-0012.1.
- Rausher, M. 1978. Search image for leaf shape in a butterfly. *Science* **200**(4345): 1071–1073. doi:10.1126/science.200.4345.1071.
- Rausher, M.D., and Simms, E.L. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. *Evolution* **43**(3): 563–572. doi:10.1111/j.1558-5646.1989.tb04252.x.
- Reeves, J.L. 2011. Vision should not be overlooked as an important sensory modality for finding host plants. *Environ. Entomol.* **40**(4): 855–63. doi:10.1603/EN10212.
- Ricciardi, A., Hoopes, M.F., Marchetti, M.P., and Lockwood, J.L. 2013. Progress toward understanding the ecological impacts of nonnative species. *Ecol. Monogr.* **83**(3): 263–282. doi:10.1890/13-0183.1.
- Riipi, M., Alatalo, R. V., Lindström, L., and Mappes, J. 2001. Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature* **413**(6855): 512–514. doi:10.1038/35097061.
- Rivero-Lynch, A.P., Brown, V.K., and Lawton, J.H. 1996. The impact of leaf shape on the feeding preference of insect herbivores: experimental and field studies with *Capsella* and *Phyllotreta*. *Philos. Trans. R. Soc. B Biol. Sci.* **351**(1348): 1671–1677. doi:10.1098/rstb.1996.0149.
- Roper, T.J., and Redston, S. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. *Anim. Behav.* **35**(3): 739–747. doi:10.1016/S0003-3472(87)80110-0.
- Rogers, G., Walker, S., and Lee, B. 2005. The role of disturbance in dryland New Zealand: past and present. In *Science for Conservation*. Available from <http://www.conservancy.org.nz/Documents/science-and-technical/SFC258.pdf>.
- Rohlf, F.J., and Marcus, L.F. 1993. A revolution in morphometrics. *Trends Ecol. Evol.* **8**(4): 129–132. doi:10.1016/0169-5347(93)90024-J.

- Roth-Nebelsick, A. 2001. Computer-based analysis of steady-state and transient heat transfer of small-size leaves by free mixed convection. *Plant, Cell Environ.* **24**(6): 631–640. doi:10.1046/j.1365-3040.2001.00712.x.
- Rowe, C. 1999. Receiver psychology and evolution of multicomponent signals. *Anim. Behav.* **58**(5): 921–931. doi:10.1006/anbe.1999.1242.
- Rowe, C., and Halpin, C. 2013. Why are warning displays multimodal? *Behav. Ecol. Sociobiol.* **67**(9): 1425–1439. doi:10.1007/s00265-013-1515-8.
- Rowland, H.M., Ihalainen, E., Lindström, L., Mappes, J., and Speed, M.P. 2007. Co-mimics have a mutualistic relationship despite unequal defences. *Nature* **448**(7149): 64–67. doi:10.1038/nature05899.
- Royer, D.L., Meyerson, L.A., Robertson, K.M., and Adams, J.M. 2009. Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PLoS One* **4**(10): e7653. doi:10.1371/journal.pone.0007653.
- Royer, D.L., and Wilf, P. 2006. Why do toothed leaves correlate with cold climates? Gas exchange at leaf margins provides new insights into a classic paleotemperature proxy. *Int. J. Plant Sci.* **167**(1): 11–18. doi:10.1086/497995.
- Rubino, D.L., and McCarthy, B.C. 2004. Presence of aposematic (warning) coloration in vascular plants of southeastern Ohio. *J. Torrey Bot. Soc.* **131**(3): 252–256. doi:10.2307/4126955.
- Ruxton, G.D., Sherratt, T.N., and Speed, M.P. 2004. *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford University Press, New York.
- Ryan, M.J., and Keddy-Hector, A.C. 1992. Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* **139**: S4–S35. doi:10.1086/285303.
- Schaefer, H.M., and Rolshausen, G. 2005. Plants on red alert: do insects pay attention? *BioEssays* **28**(1): 65–71. doi:10.1002/bies.20340.
- Schaefer, H.M., and Ruxton, G.D. 2009. Deception in plants: mimicry or perceptual exploitation? *Trends Ecol. Evol.* **24**(12): 676–685. doi:10.1016/j.tree.2009.06.006.

- Schaefer, H.M., and Ruxton, G.D. 2011. Plant-animal communication. Oxford University Press, Oxford.
- Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M., Schulze, E.D., Roscher, C., Weigelt, A., Allan, E., Beler, H., Bonkowski, M., Buchmann, N., Buscot, F., Clement, L.W., Ebeling, A., Engels, C., Halle, S., Kertscher, I., Klein, A.M., Koller, R., König, S., Kowalski, E., Kummer, V., Kuu, A., Lange, M., Lauterbach, D., Middelhoff, C., Migunova, V.D., Milcu, A., Müller, R., Partsch, S., Petermann, J.S., Renker, C., Rottstock, T., Sabais, A., Scheu, S., Schumacher, J., Temperton, V.M., and Tschardt, T. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **468**(7323): 553–556. doi:10.1038/nature09492.
- Schlaepfer, M.A., Sax, D.F., and Olden, J.D. 2011. The potential conservation value of non-native species. *Conserv. Biol.* **25**(3): 428–437. doi:10.1111/j.1523-1739.2010.01646.x.
- Schmidt, R.S. 1958. Behavioural evidence on the evolution of Batesian mimicry. *Anim. Behav.* **6**(3–4): 129–138. doi:10.1016/0003-3472(58)90042-3.
- Schoonhoven, L.M., van Loon, J.J.A., and Dicke, M. 2005. Insect–Plant Biology. Oxford University Press, Oxford, UK.
- Schröder, R., and Hilker, M. 2008. The relevance of background odor in resource location by insects: a behavioral approach. *Bioscience* **58**(4): 308–316. doi:10.1641/B580406.
- Scoffoni, C., Rawls, M., McKown, A., Cochard, H., and Sack, L. 2011. Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. *Plant Physiol.* **156**(2): 832–843. doi:10.1104/pp.111.173856.
- Searle, K.R., and Shipley, L.A. 2008. The comparative feeding behaviour of large browsing and grazing herbivores. *In* The Ecology of Browsing and Grazing. Edited by I.J. Gordon and H.H.T. Prins. Springer, Berlin. pp. 117–148.
- Sheppard, P.M. 1959. The evolution of mimicry: a problem in ecology and genetics. *Cold Spring Harb. Symp. Quant. Biol.* **24**: 131–140. doi:10.1101/SQB.1959.024.01.013.
- Sherratt, T.N. 2001. The evolution of female-limited polymorphisms in damselflies: a signal detection model. *Ecol. Lett.* **4**(1): 22–29. doi:10.1046/j.1461-0248.2001.00184.x.

- Sherratt, T.N. 2002. The evolution of imperfect mimicry. *Behav. Ecol.* **13**(6): 821–826. doi:10.1093/beheco/13.6.821.
- Sherratt, T.N., and Beatty, C.D. 2003. The evolution of warning signals as reliable indicators of prey defense. *Am. Nat.* **162**(4): 377–389. doi:10.1086/378047.
- Sherratt, T.N., Whissell, E., Webster, R., and Kikuchi, D.W. 2015. Hierarchical overshadowing of stimuli and its role in mimicry evolution. *Anim. Behav.* **108**: 73–79. doi:10.1016/j.anbehav.2015.07.011.
- Shimshi, D. 1979. Two ecotypes of *Iris atrofusca* Bak. and their relationship to man-modified habitats. *Isr. J. Bot.* **28**(2): 80–86. doi:10.1080/0021213X.1979.10676859.
- Shipley, L.A., Forbey, J.S., and Moore, B.D. 2009. Revisiting the dietary niche: when is a mammalian herbivore a specialist. *Integr. Comp. Biol.* **49**(3): 274–290. doi:10.1093/icb/icp051.
- Siefermann-Harms, D. 1985. Carotenoids in photosynthesis. I. Location in photosynthetic membranes and light-harvesting function. *Biochim. Biophys. Acta* **811**(4): 325–355. doi:10.1016/0304-4173(85)90006-0.
- Sih, A., and Christensen, B. 2001. Optimal diet theory: when does it work, and when and why does it fail? *Anim. Behav.* **61**(2): 379–390. doi:10.1006/anbe.2000.1592.
- Sih, A., Englund, G., and Wooster, D. 1998. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* **13**(9): 350–355. doi:10.1016/S0169-5347(98)01437-2.
- Sillén-Tullberg, B. 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia* **67**(3): 411–415. doi:10.1007/BF00384948.
- Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., and Raubenheimer, D. 2004. Optimal foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**(6): 1299–1311. doi:10.1016/j.anbehav.2004.03.003.
- Sinclair, C., and Hoffmann, A.A. 2003. Monitoring salt stress in grapevines: Are measures of plant trait variability useful? *J. Appl. Ecol.* **40**(5): 928–937. doi:10.1046/j.1365-2664.2003.00843.x.

- Singer, M.S., Bernays, E.A., and Carrière, Y. 2002. The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Anim. Behav.* **64**(4): 629–643. doi:10.1006/anbe.2002.3082.
- Skelhorn, J., Halpin, C.G., and Rowe, C. 2016. Learning about aposematic prey. *Behav. Ecol.* **27**(4): 955–964. doi:10.1093/beheco/arw009.
- Skelhorn, J., and Rowe, C. 2005. Frequency-dependent taste-rejection by avian predation may select for defence chemical polymorphisms in aposematic prey. *Biol. Lett.* **1**(4): 500–503. doi:10.1098/rsbl.2005.0359.
- Skelhorn, J., and Rowe, C. 2006a. Predator avoidance learning of prey with secreted or stored defences and the evolution of insect defences. *Anim. Behav.* **72**(4): 827–834. doi:10.1016/j.anbehav.2005.12.010.
- Skelhorn, J., and Rowe, C. 2006b. Prey palatability influences predator learning and memory. *Anim. Behav.* **71**(5): 1111–1118. doi:10.1016/j.anbehav.2005.08.011.
- Skelhorn, J., and Rowe, C. 2007. Predators' toxin burdens influence their strategic decisions to eat toxic prey. *Curr. Biol.* **17**(17): 1479–1483. doi:10.1016/j.cub.2007.07.064.
- Skelhorn, J., Rowland, H.M., and Ruxton, G.D. 2009. The evolution and ecology of masquerade. *Biol. J. Linn. Soc.* **99**(1): 1–8. doi:10.1111/j.1095-8312.2009.01347.x.
- Skelhorn, J., Rowland, H.M., Speed, M.P., and Ruxton, G.D. 2010. Masquerade: camouflage without crypsis. *Science* **327**(5961): 51. doi:10.1126/science.1181931.
- Soltau, U., Dötterl, S., and Liede-Schumann, S. 2008. Leaf variegation in *Caladium steudneriifolium* (Araceae): a case of mimicry? *Evol. Ecol.* **23**(4): 503–512. doi:10.1007/s10682-008-9248-2.
- Speed, M.P., and Ruxton, G.D. 2010. Imperfect Batesian mimicry and the conspicuousness costs of mimetic resemblance. *Am. Nat.* **176**(1): E1–14. doi:10.1086/652990.
- Spence, C., Nicholls, M.E., Gillespie, N., and Driver, J. 1998. Cross-modal links in exogenous covert spatial orienting between touch, audition, and vision. *Percept. Psychophys.* **60**(4): 544–557. doi:10.3758/BF03206045.
- Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.* **78**(1): 23–55. doi:10.1086/367580.

- Stephens, D.W., and Krebs, J.R. 1986. Foraging theory. Princeton University Press, Princeton, NJ.
- Stevens, M., and Merilaita, S. 2009a. Animal camouflage: current issues and new perspectives. *Philos. Trans. R. Soc. B Biol. Sci.* **364**(1516): 423–427. doi:10.1098/rstb.2008.0217.
- Stevens, M., and Merilaita, S. 2009b. Defining disruptive coloration and distinguishing its functions. *Philos. Trans. R. Soc. B Biol. Sci.* **364**(1516): 481–8. doi:10.1098/rstb.2008.0216.
- Strauss, S.Y., and Agrawal, A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* **14**(5): 179–185. doi:10.1016/S0169-5347(98)01576-6.
- Strauss, S.Y., Rudgers, J.A., Lau, J.A., and Irwin, R.E. 2002. Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.* **17**(6): 278–285. doi:10.1016/S0169-5347(02)02483-7.
- Strayer, D.L. 2012. Eight questions about invasions and ecosystem functioning. *Ecol. Lett.* **15**(10): 1199–1210. doi:10.1111/j.1461-0248.2012.01817.x.
- Sumby, W.H., and Pollack, I. 1954. Visual contribution to speech intelligibility in noise. *J. Acoust. Soc. Am.* **26**(2): 212–215. doi:dx.doi.org/10.1121/1.1907309.
- Summers, K., Speed, M.P., Blount, J.D., and Stuckert, A.M.M. 2015. Are aposematic signals honest? A review. *J. Evol. Biol.* **28**(9): 1583–1599. doi:10.1111/jeb.12676.
- Sword, G.A. 2002. A role for phenotypic plasticity in the evolution of aposematism. *Proc. R. Soc. B Biol. Sci.* **269**(1501): 1639–1644. doi:10.1098/rspb.2002.2060.
- Szallasi, Á., Jonassohn, M., Acs, G., Bíró, T., Acs, P., Blumberg, P.M., and Sterner, O. 1996. The stimulation of capsaicin-sensitive neurones in a vanilloid receptor-mediated fashion by pungent terpenoids possessing an unsaturated 1,4-dialdehyde moiety. *Br. J. Pharmacol.* **119**(2): 283–290. doi:10.1111/j.1476-5381.1996.tb15983.x.
- Tanentzap, A.J., Lee, W.G., Dugdale, J.S., Patrick, B.P., Fenner, M., Walker, S., and Coomes, D.A. 2011. Differential responses of vertebrate and invertebrate herbivores to traits of New Zealand subalpine shrubs. *Ecology* **92**(4): 994–999. doi:10.1890/10-0861.1.

- Terashima, I., Fujita, T., Inoue, T., Chow, W.S., and Oguchi, R. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *Plant Cell Physiol.* **50**(4): 684–697. doi:10.1093/pcp/pcp034.
- Thoday, D. 1931. The significance of reduction in the size of leaves. *J. Ecol.* **19**(2): 297–303. doi:10.2307/2255823.
- Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S., and Schnitzler, J.P. 2006. Practical approaches to plant volatile analysis. *Plant J.* **45**(4): 540–560. doi:10.1111/j.1365-313X.2005.02612.x.
- Tinbergen, N. 1952. “Derived” activities; their causation, biological significance, origin, and emancipation during evolution. *Q. Rev. Biol.* **27**(1): 1–32. doi:10.1086/398642.
- Troje, N.F., Huber, L., Loidolt, M., Aust, U., and Fieder, M. 1999. Categorical learning in pigeons: the role of texture and shape in complex static stimuli. *Vision Res.* **39**(2): 353–366. doi:10.1016/S0042-6989(98)00153-9.
- Tsukaya, H. 2005. Leaf shape: genetic controls and environmental factors. *Int. J. Dev. Biol.* **49**(5-6): 547–555. doi:10.1387/ijdb.041921ht.
- Tullberg, B.S., Merilaita, S., and Wiklund, C. 2005. Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. *Proc. R. Soc. B Biol. Sci.* **272**(1570): 1315–1321. doi:10.1098/rspb.2005.3079.
- Tylianakis, J.M., Didham, R.K., Bascompte, J., and Wardle, D.A. 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* **11**(12): 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x.
- Uchikawa, K., and Ikeda, M. 1981. Temporal deterioration of wavelength discrimination with successive comparison method. *Vision Res.* **21**(4): 591–595. doi:10.1016/0042-6989(81)90106-1.
- Ulrich, S., and Brady, P. 2003. Monitoring terrestrial habitats in Wellington Conservancy. A strategy for 2003-2012. Department of Conservation, Wellington.

- Valkonen, J., Niskanen, M., Björklund, M., and Mappes, J. 2011. Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evol. Ecol.* **25**(5): 1047–1063. doi:10.1007/s10682-011-9463-0.
- Vane-Wright, R.I. 1980. On the definition of mimicry. *Biol. J. Linn. Soc.* **13**(1): 1–6. doi/10.1111/j.1095-8312.1980.tb00066.x.
- Varnamkhasti, A.S., Milchunas, D.G., Lauenroth, W.K., and Goetz, H. 1995. Production and rain use efficiency in short-grass steppe: grazing history, defoliation and water resource. *J. Veg. Sci.* **6**(6): 787–796. doi:10.2307/3236392.
- Vereecken, N.J., and Schiestl, F.P. 2008. The evolution of imperfect floral mimicry. *Proc. Natl. Acad. Sci. U. S. A.* **105**(21): 7484–7488. doi:10.1073/pnas.0800194105.
- Villalba, J.J., and Provenza, F.D. 2000. Roles of novelty, generalization, and postingestive feedback in the recognition of foods by lambs. *J. Anim. Sci.* **78**(12): 3060–3069. doi:10.2527/2000.78123060x.
- Villalba, J.J., and Provenza, F.D. 2005. Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. *J. Chem. Ecol.* **31**(1): 123–138. doi:10.1007/s10886-005-0979-z.
- Villalba, J.J., and Provenza, F.D. 2009. Learning and dietary choice in herbivores. *Rangel. Ecol. Manag.* **62**(5): 399–406. doi:10.2111/08-076.1.
- Vitule, J.R.S., Freire, C.A., Vazquez, D.P., Nuñez, M.A., and Simberloff, D. 2012. Revisiting the potential conservation value of non-native species. *Conserv. Biol.* **26**(6): 1153–1155. doi:10.1111/j.1523-1739.2012.01950.x.
- Vogel, S. 1968. “Sun leaves” and “shade leaves”: differences in convective heat dissipation. *Ecology* **49**(6): 1203–1204. doi:10.2307/1934517.
- Vourc’h, G., Garine-Wichatitsky, M. De, Labbé, A., Rosolowski, D., Martin, J.L., and Fritz, H. 2002. Monoterpene effect on feeding choice by deer. *J. Chem. Ecol.* **27**(12): 2411–2427. doi:10.1023/A:1021423816695.
- Wallace, A.R. 1867. Proceedings of the entomological society of London. *In* Transactions of the Entomological Society of London. London.

- Wallace, A.R. 1889. Darwinism: an exposition of the theory of natural selection, with some of its applications. Macmillian, London.
- Wang, G., Hobbs, N.T., Boone, R.B., Illius, A.W., Gordon, I.J., Gross, J.E., and Hamlin, K.L. 2006. Spatial and temporal variability modify density dependence in populations of large herbivores. *Ecology* **87**(1): 95–102. doi:10.1890/05-0355.
- Weight, C., Parnham, D., and Waites, R. 2008. LeafAnalyser: a computational method for rapid and large-scale analyses of leaf shape variation. *Plant J.* **53**(3): 578–586. doi:10.1111/j.1365-313X.2007.03330.x.
- Werner, E.E., and Hall, D.J. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology* **55**(5): 1042–1052. doi:10.2307/1940354.
- Werner, S.J., and Provenza, F.D. 2011. Reconciling sensory cues and varied consequences of avian repellents. *Physiol. Behav.* **102**(2): 158–163. doi:10.1016/j.physbeh.2010.10.012.
- Wheeler, K.G.R. 2004. A natural history of nettles. Trafford, Victoria.
- Whittaker, R.H., and Feeny, P.P. 1971. Allelochemicals: chemical interactions between species. *Science* **171**(3973): 757–770. doi:10.1126/science.171.3973.757.
- Wickler, W. 1968. Mimicry in plants and animals (Translated from the German by R. D. Martin). Weidenfeld & Nicolson, London.
- Williams, P. 2007. The distribution of bumblebee colour patterns worldwide: Possible significance for thermoregulation, crypsis, and warning mimicry. *Biol. J. Linn. Soc.* **92**(1): 97–118. doi:10.1111/j.1095-8312.2007.00878.x.
- Williamson, M., and Fitter, A. 1996. The varying success of invaders. *Ecology* **77**(6): 1661–1666. doi:10.2307/2265769.
- Wilson, S.L., and Kerley, G.I.H. 2003. Bite diameter selection by thicket browsers: the effect of body size and plant morphology on forage intake and quality. *For. Ecol. Manage.* **181**(1-2): 51–65. doi:10.1016/S0378-1127(03)00114-2.
- Wood, J.R., Rawlence, N.J., Rogers, G.M., Austin, J.J., Worthy, T.H., and Cooper, A. 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand

- megaherbivore moa (Aves, Dinornithiformes). *Quat. Sci. Rev.* **27**(27-28): 2593–2602. Elsevier Ltd. doi:10.1016/j.quascirev.2008.09.019.
- Wood, J.R., Wilmschurst, J.M., Richardson, S.J., Rawlence, N.J., Wagstaff, S.J., Worthy, T.H., and Cooper, A. 2013. Resolving lost herbivore community structure using coprolites of four sympatric moa species (Aves: Dinornithiformes). *Proc. Natl. Acad. Sci. U. S. A.* **110**(42): 16910–16915. doi:10.1073/pnas.1307700110.
- Wood, J.R., Wilmschurst, J.M., Wagstaff, S.J., Worthy, T.H., Rawlence, N.J., and Cooper, A. 2012. High-resolution coproecology: using coprolites to reconstruct the habits and habitats of New Zealand's extinct upland moa (*Megalapteryx didinus*). *PLoS One* **7**(6): e40025. doi:10.1371/journal.pone.0040025.
- Worthy, T.H., and Holdaway, R.N. 2002. *The Lost World of the Moa: Prehistoric Life of New Zealand*. Canterbury University Press, Christchurch.
- Xiao, F., and Cuthill, I.C. 2016. Background complexity and the detectability of camouflaged targets by birds and humans. *Proc. R. Soc. B Biol. Sci.* **283**(1838): 20161527. doi:10.1098/rspb.2016.1527.
- Yager, K.G., Schaefer, H.M., and Gould, K.S. 2016. The significance of shared leaf shape in *Alseuosmia pusilla* and *Pseudowintera colorata*. *Botany* **94**(7): 555–564. doi:10.1139/cjb-2016-0049.
- Yamasaki, T., Sato, M., and Sakoguchi, H. 1997. (-)-Germacrene D: Masking substance of attractants for the cerambycid beetle, *Monochamus alternatus* (Hope). *Appl. Entomol. Zool.* **32**(3): 423–429. doi:10.1303/aez.32.423.
- Yamasue, Y. 2001. Strategy of *Echinochloa oryzicola* Vasing. for survival in flooded rice. *Weed Biol. Manag.* **1**(1): 28–36. doi:10.1046/j.1445-6664.2001.00008.x.
- Yosef, R., and Whitman, D.W. 1992. Predator exaptations and defensive adaptations in evolutionary balance: no defence is perfect. *Evol. Ecol.* **6**(6): 527–536. doi:10.1007/BF02270696.
- Zhang, J., and Mueller, S.T. 2005. A note on ROC analysis and non-parametric estimate of sensitivity. *Psychometrika* **70**(1): 203–212. doi:10.1007/s11336-003-1119-8.

Zwieniecki, M.A., Boyce, C.K., and Holbrook, N.M. 2004. Hydraulic limitations imposed by crown placement determine final size and shape of *Quercus rubra* L. leaves. Plant, Cell Environ. **27**(3): 357–365. doi:10.1111/j.1365-3040.2003.01153.x.