

Are there pollination syndromes in the Australian epacrids (Ericaceae: Styphelioideae)? A novel statistical method to identify key floral traits per syndrome

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Received: 11 November 2012 Revision requested: 25 January 2013 Accepted: 3 April 2013 Published electronically: 16 May 2013

- **Background and Aims** Convergent floral traits hypothesized as attracting particular pollinators are known as pollination syndromes. Floral diversity suggests that the Australian epacrid flora may be adapted to pollinator type. Currently there are empirical data on the pollination systems for 87 species (approx. 15 % of Australian epacrids). This provides an opportunity to test for pollination syndromes and their important morphological traits in an iconic element of the Australian flora.
- **Methods** Data on epacrid–pollinator relationships were obtained from published literature and field observation. A multivariate approach was used to test whether epacrid floral attributes related to pollinator profiles. Statistical classification was then used to rank floral attributes according to their predictive value. Data sets excluding mixed pollination systems were used to test the predictive power of statistical classification to identify pollination models.
- **Key Results** Floral attributes are correlated with bird, fly and bee pollination. Using floral attributes identified as correlating with pollinator type, bird pollination is classified with 86 % accuracy, red flowers being the most important predictor. Fly and bee pollination are classified with 78 and 69 % accuracy, but have a lack of individually important floral predictors. Excluding mixed pollination systems improved the accuracy of the prediction of both bee and fly pollination systems.
- **Conclusions** Although most epacrids have generalized pollination systems, a correlation between bird pollination and red, long-tubed epacrids is found. Statistical classification highlights the relative importance of each floral attribute in relation to pollinator type and proves useful in classifying epacrids to bird, fly and bee pollination systems.

Key words: Epacridaceae (epacrids), Ericaceae, multivariate analysis, plant–pollinator interactions, pollination syndromes, Random Forests, statistical classification, Styphelioideae.

INTRODUCTION

Convergent floral traits hypothesized as attracting particular pollen vectors are known as pollination syndromes (Faegri and van der Pijl, 1979). The concept of pollination syndromes is both pivotal and controversial in pollination ecology. Pollination syndromes have been used to both infer the pollinators of plant species in the absence of observations and to provide a mechanistic explanation of floral diversity (Fletcher, 1977; Fenster *et al.*, 2004; Perez *et al.*, 2006; Whittall and Hodges, 2007). However, the traditional syndromes described by Faegri and van der Pijl (1979) have been shown to be unreliable predictors of major pollinators in some plant communities (Hingston and McQuillan, 2000; Ollerton *et al.*, 2009). The accuracy of these syndromes also differs across plant families, with Fabaceae, Apocynaceae and Asteraceae conforming better than other families (Ollerton *et al.*, 2009). Regional variability in floras and pollinators also means that syndromes described in one part of the world may not be present in another (Newstrom and Robertson, 2005). As a result, researchers have tailored the syndrome concept to specific geographic areas and plant taxa (Thomson *et al.*, 2000; Andersson *et al.*, 2002; Kay and Schemske, 2003; Ollerton *et al.*, 2003; Goldblatt and Manning, 2005; Castellanos *et al.*, 2006). Red,

tubular flowers have been associated with hummingbird pollination in southern Chile (Armesto *et al.*, 1996). However, bird pollination is almost absent in Europe, with the yellow-flowered legume *Anagyris foetida* being the notable exception (Cronk and Ojeda, 2008).

On Australia's southern island of Tasmania, the traditional pollination syndromes (Faegri and van der Pijl, 1979) have proved to be largely unreliable predictors of floral visitors (Hingston and McQuillan, 2000). While floral morphology suggested the presence of pollination syndromes after Faegri and van der Pijl (1979), Hingston and McQuillan (2000) found the majority of flowers unspecialized and hosting an array of visitors – very few were exclusively visited by one animal group. While many Australian plants appear to have generalized pollination systems, different animal groups may have different levels of effectiveness as pollinators within these generalized systems (Fenster *et al.*, 2004). Thus, pollination syndromes may be present even when a plant is visited by multiple animal groups, such as birds and insects (Wilson *et al.*, 2004).

There is evidence to suggest the Australian flora has undergone adaptation in relation to pollinator type, particularly in relation to the honeyeaters (Meliphagidae), which may exert a similar influence to that of hummingbirds on the American flora (Paton and Ford, 1977; Hingston and McQuillan, 2000;

Johnson *et al.*, 2010). To date, there has been no widespread search for pollination syndromes specific to the Australian region.

The Australian epacrid species (Styphelioideae, Ericaceae) were selected as a case study for examining the correlation between floral features and pollinator assemblages because of their large species number (>500 species), high floral diversity (Stephens *et al.*, 2004), prominence in the Australian landscape and rich history of pollinator observations (Fletcher, 1977; Paton and Ford, 1977; Ford *et al.*, 1979; Green and Osborne, 1994; Corbett, 1995; Keighery, 1996; Higham and McQuillan, 2000; Hingston and McQuillan, 2000; Houston, 2000; Houston and Ladd, 2002; Celebrezze and Paton, 2004; Johnson *et al.*, 2010, 2011; Schneemilch *et al.*, 2011). The epacrids consist of woody plants ranging from small prostrate shrubs to temperate rainforest emergents. Their range extends across south-east Asia, Oceania, New Zealand, New Caledonia and Tierra del Fuego, but most of the species and taxonomic diversity in the subfamily is found in Australia. Although all epacrid flowers are actinomorphic, there is much variety in size and colour, from small white flowers in *Leucopogon* to large red tubular flowers in *Prionotes*; from *Richea* species that abscise their corollas to present a brush-like flower to the small green, inconspicuous flowers of *Acrotriche* (Fig. 1). Currently there are empirical data on the pollination systems for 87 epacrid species (approx. 15% of Australian epacrids) across six of the seven tribes.

This provides an opportunity to test for pollination syndromes in an iconic element of the Australian flora.

Given that floral features may function in an integrated fashion and be selected as a group (Castellanos *et al.*, 2004), a multivariate approach is used to test the hypothesis that epacrid floral attributes are correlated with pollinator type. In addressing this hypothesis, an ensemble classifier, Random Forests (RF) (Breiman, 2001), is used to explore for suites of floral traits (pollination syndromes) predictive of pollinator types. Statistical classification procedures such as RF which are capable of modelling complex interactions among predictor variables are widely used in the broader field of ecology (Cutler *et al.*, 2007) but are yet to be explored in the context of pollination ecology.

METHODS

Pollinator profiles

Information on floral visitors to 87 epacrid species came from the published literature and field observations (Supplementary Data Tables S1, S2). *Acrotriche* species were excluded from analyses due to uncertainty regarding their pollinators (Johnson *et al.*, 2011; Schneemilch *et al.*, 2011). To standardize data from different sources, the term ‘potential pollinator’ (hereafter synonymous with ‘pollinator’) was used to describe an animal observed to interact with epacrid flowers. Thus, a flower visitor was

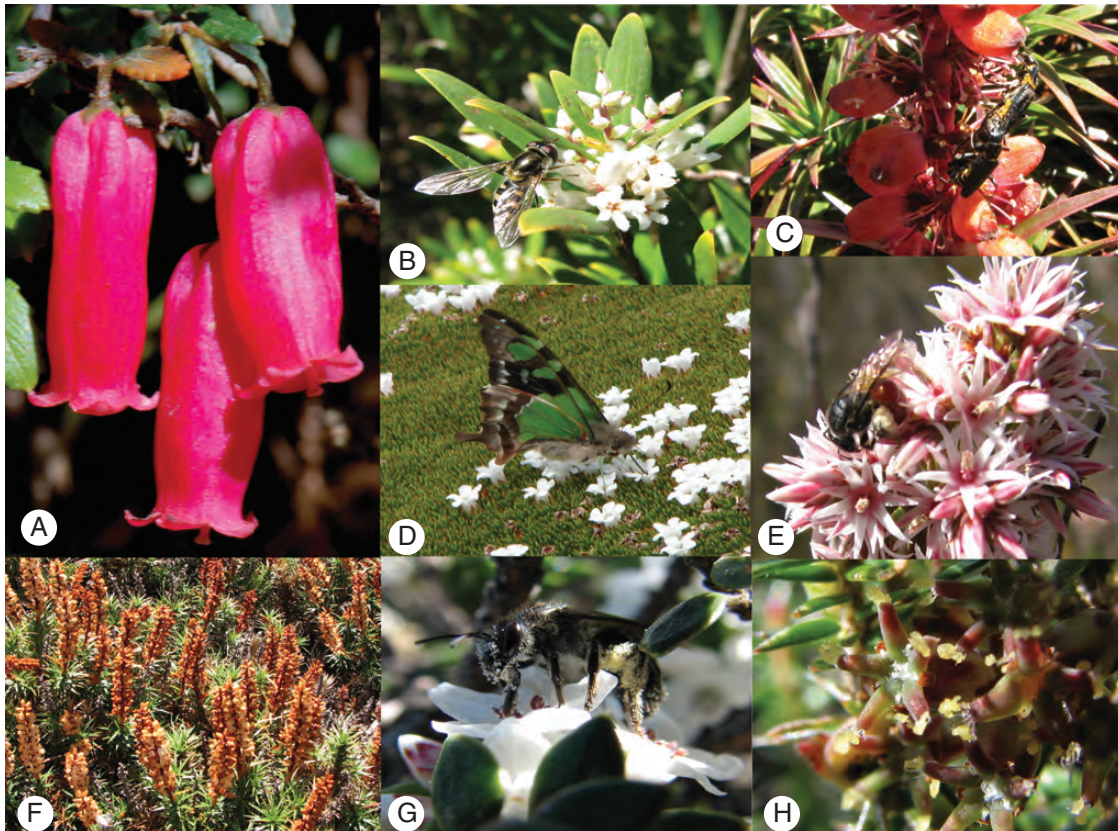


FIG. 1. Epacrid floral diversity. (A) Bird-pollinated flowers of *Prionotes*. (B) Hoverfly (Syrphidae) on *Leucopogon parviflorus*. (C) Beetles (*Chauliognathus tricolor*) on *Richea scoparia*. (D) Macleay's swallowtail butterfly (*Graphium macleayanum*) visiting *Dracophyllum minimum*. (E) A bee [*Lasioglossum* (*Parasphecodes*)] collecting pollen by sonication from *Sprengelia incarnata*. (F) *Richea scoparia* – orange flowers are rare in epacrids. (G) A bee (*Leioproctus* sp.) collecting pollen from *Epacris marginata*. (H) *Acrotriche serrulata* flowers with secondary pollen presentation.

considered a pollinator only when it was described in the literature as ‘foraging at flowers, brushing anthers and stigma’ and/or ‘carrying epacrid pollen’. Where generalized pollination systems are reported, there is often no or limited information on the effectiveness of each pollinator type. Thus, for the purpose of the current work, all pollinators have been treated as equally effective. To analyse and interpret plant and animal relationships, species-level identification was required for plants. For animals, the requirement was data making it possible to categorize them into functional groups based on Fenster *et al.* (2004) – bee (Hymenoptera), fly (Diptera), beetle (Coleoptera), butterfly (Lepidoptera), moth (Lepidoptera), wasp (Hymenoptera) and bird (Aves) (Supplementary Data Table S2).

Pollinator information was collated using Google Scholar (most recently 28 March 2012) searches for the key words ‘Pollinat* Epacridaceae’ or ‘Pollinat* Styphelioideae’. The words ‘Pollination’, ‘Epacridaceae’ and ‘Styphelioideae’ were also used to search within journals that routinely publish on pollination ecology. Each study had its own agenda; therefore, completeness of pollinator profiles may be variable and may not cover all pollination scenarios in epacrids (e.g. there are few nocturnal pollination studies). Thus, the data set should be considered non-random.

Floral trait profiles

Information on four floral traits for all 87 epacrid species where pollinators were known was obtained from the literature (Allan, 1961; Curtis, 1963; Laing and Blackwell, 1964; Van Royen, 1982; Jessop and Toelken, 1986; Mark and Adams, 1986; Harden, 1992; Corrick *et al.*, 1996; Walsh and Entwisle, 1996; Brown and Streiber, 1999; Packowska and Chapman, 2000; Wheeler *et al.*, 2002; FloraBase, <http://florabase.dec.wa.gov.au/>). Only attributes for which data were accessible for all 87 plant species were included. Thus the attributes concentrate on display colour (seven categories), flower type including length of floral tube (five categories), width of corolla mouth (three categories) and presence of nectar. This means a slight weighting towards display colour in the analyses. However, all these attributes have previously been useful in delineating bird-pollinated from insect-pollinated flowers (Faegri and van de Pijl, 1979; Fenster *et al.*, 2004; Wilson *et al.*, 2004).

Traits were scored as 1 if present and 0 if not. Display colour was categorized as blue/purple, red, pink, yellow, green and white (scored as red corolla = 1 or not red corolla = 0). Colour usually referred to the corolla. However, corolla-abscising *Richea* taxa present like a brush flower and previous research suggests that animal visitation occurs after corolla abscission (Johnson, 2012). Thus, stamen colour rather than corolla colour was scored. Flower shape was scored as cup/bell, brush, long tube (10+ mm), medium tube (5 to <10 mm) and short tube (<5 mm). Corolla mouth was recorded as ‘constricted’ (generally <1 mm) where the corolla was nearly closed at the top such as in *Astroloma* species, ‘narrow’ (generally 1 to <2.5 mm) where it tapered in near the top, and ‘wide’ (generally >2.5 mm) where it tended to be at least as wide as the rest of the floral tube.

Data analysis

Ordination of floral attributes and pollinator profiles. Two non-metric multi-dimensional Scaling (NMDS) analyses were

performed on epacrid floral and pollinator information using the ecodist library in R 2.15.1 (Goslee and Urban, 2007): (1) epacrid species based on floral characters; and (2) epacrid species based on pollinator profiles (Supplementary Data Tables S3, S4). A Bray–Curtis dissimilarity matrix was used as input to the pollinator ordination as many of the cells in the data matrix contained zeros. A Euclidean dissimilarity matrix was used as input to the floral ordination. The Bray–Curtis dissimilarity matrix puts less weight on shared absences than a Euclidean dissimilarity matrix. Pollinator information originated from many sources and it is probable that in some cases a zero reflects a lack of information rather than absence of a pollinator. In the case of the epacrids, knowledge of floral attributes is much more complete than that of pollinators, thus a zero represents a legitimate absence of an attribute.

Vectors indicating the direction and strength of (1) pollinators in floral ordination space and (2) floral attributes in pollinator ordination space, were fitted. In both cases, the null hypothesis was that the strength of the relationship between floral attributes and pollinator profiles was more than can be expected by chance, $P \leq 0.05$ (P -values were based on 10 000 permutations).

Only native pollinators were included in the analyses. The two introduced species, honeybee *Apis mellifera* and bumblebee *Bombus terrestris*, were excluded. The honeybee [introduced to Australia in the early 19th century (Doull, 1973)] and bumblebee [introduced in 1992 (Semmens, 1993)] are not part of the historical pollinator assemblage and are unlikely to have had time to effect evolutionary change. Lizards were excluded due to uncertainty about their role as either pollinators or nectar-robbers of *Richea scoparia* (Olsson *et al.*, 2000; Johnson, 2012).

Correlations between floral attribute and pollinator dissimilarity matrices. The Mantel test in the ecodist package in R 2.2.1 (Goslee and Urban, 2007) was used to test whether the Bray–Curtis dissimilarity matrix based on pollinators was correlated with the Euclidean matrix based on floral traits (P -values are for the two-tailed test and were based on 10 000 permutations).

The Mantel test was used (in the ecodist package in R) to determine whether there was any association between a Euclidean matrix based on floral attributes and a matrix containing 0s for pairs of epacrids with a particular pollinator (e.g. birds) in their profiles or pairs of epacrids without, and 1s otherwise. The analysis was repeated for each pollinator group.

Identifying pollination syndromes. Statistical classification was used to obtain a set of floral attributes that would be predictive of the pollinator groups that were found to be related to floral attributes by the above analyses. Random Forests (Breiman, 2001) is a classifier capable of modelling complex interactions among predictor variables. It is not a tool for hypothesis testing (Cutler *et al.*, 2007); rather, the variable importance measure it gives (explained below) is useful to identify the floral attributes that are important for particular pollinator groups.

Random Forests builds a ‘forest’ of classification trees based on random sub-sets of the data, using randomly restricted and selected predictors (floral attributes) for each of the splits in the trees (Strobl *et al.*, 2008). Strobl *et al.* (2009) suggest that this enables a better examination of the contribution and behaviour of each predictor, particularly when compared with simpler

models (such as simple or mixed effect regression models). In addition, results from numerous classification trees have been found to be superior to that of one classification tree (Strobl *et al.*, 2008). Random Forests was run using the randomForest 4.6-6 library in R2.15.1 (Breiman, 2001; Liaw and Wiener, 2002).

Random Forests was used to build floral classification models for the statistically significant pollinator groups from the floral attributes and pollinator tables (Supplementary Data Tables S3, S4). The RF program built 500 classification trees, with four predictors (floral attributes) of pollinator type tried at each split. To determine a classification for each epacrid, the classifier votes over each of the 500 random trees and the majority decision is returned. About 63 % of the original observations occur in each bootstrap sample. A particular strength of the RF technique is that the observations not used in a particular bootstrap sample (the remaining 37 %) constitute the out-of-bag (OOB) observations and can be used to estimate both classification error and the importance of each variable (Cutler *et al.*, 2007). Variable importance is measured by randomly permuting the OOB observations and passing them down the trees to get new predictions. It is the difference between the misclassification rate for the randomly permuted OOB and the original OOB data, divided by the standard error (Cutler *et al.*, 2007). This process returns a relative ranking of the variable importance of the floral traits in predicting a particular pollinator type. Thus, the floral classifications (or pollination syndromes) obtained here relate specifically to the 87 epacrid species. Predicted outcomes (based on the randomly permuted OOB data) and the known data (original OOB data) were tabulated.

To test the accuracy of bird, fly and bee RF classification models, each model was used to classify pollination systems from the floral traits data set that was used to build them (Supplementary Data Table 5).

To determine the effects of generalized pollination systems on model accuracy, RF floral classification models were also built on modified data sets (Supplementary Data Table S6, Fig. S2): (1) the three epacrids pollinated by both birds and insects were removed to observe the effect on bird pollination model accuracy; and (2) the 23 epacrids pollinated by both flies and bees were removed to observe the effects on the accuracy of both these models.

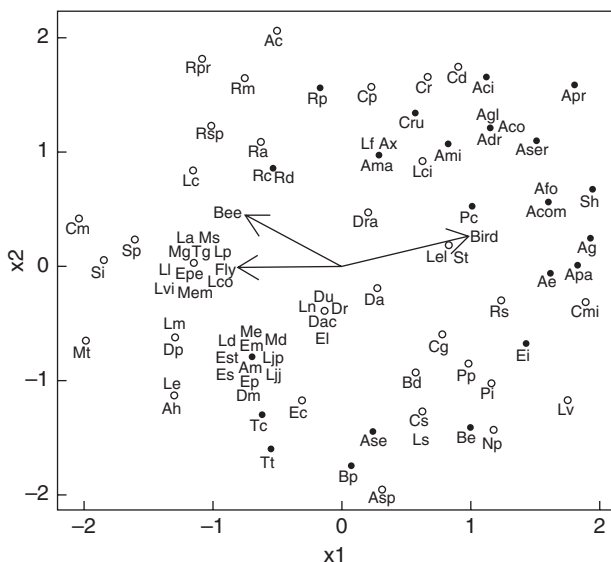
RESULTS

Pollinator profiles

Information on biotic pollinators was available for 87 epacrid species representing 22 genera and six tribes (Fig. 1; Supplementary Data Table S2). About 41 % of epacrids sampled had bees in their profiles, 35 % flies, 29 % birds, 26 % butterflies, 22 % beetles, 15 % moths and 6 % wasps. A total of 63 % of all species were pollinated by only one functional group of pollinators (40 % by birds, 15 % by flies, 15 % by butterflies, 13 % by bees, 9 % by moths, 9 % by beetles and 0 % by wasps). Thirty-five per cent used more than one functional group of pollinators: only 3 % of taxa had both birds and insects in their pollinator profiles; and 26 % had both bees and flies in their profiles. Flies and bees accounted for the greatest number of pollinating taxa and visited the most plant species.

Ordination of floral attributes and pollinator profiles

Three pollinator groups were related to the variation in floral ordination space (Fig. 2; see Appendix); from strongest to weakest correlation: bird, $r = 0.527$, $P = 0.0001$; bee, $r = 0.445$, $P = 0.0001$; and fly, $r = 0.416$, $P = 0.0005$. The vector for bird pollination was opposed to the vector for fly pollination and roughly for bee pollination.



Code	Species' name	Code	Species' name	Code	Species' name
Ac	<i>Acrotriche cordata</i>	Cs	<i>Cyathodes straminea</i>	Lp	<i>Leucopogon parviflorus</i>
Aci	<i>Astroloma ciliatum</i>	Da	<i>Dracophyllum acerorum</i>	Ls	<i>Lissanthe strigosa</i>
Aco	<i>Astroloma conostephioides</i>	Dac	<i>Dracophyllum acicularifolium</i>	Lv	<i>Leucopogon verticillatus</i>
Acom	<i>Astroloma compactum</i>	Dm	<i>Dracophyllum minimum</i>	Lvi	<i>Leucopogon virgatus</i>
Adr	<i>Astroloma drummondii</i>	Dp	<i>Dracophyllum pronum</i>	Md	<i>Montitega dealbata</i>
Ae	<i>Astroloma epacridis</i>	Dr	<i>Dracophyllum rosmarinifolium</i>	Me	<i>Monotoca elliptica</i>
Afo	<i>Astroloma foliosum</i>	Dra	<i>Dracophyllum ramosum</i>	Mem	<i>Monotoca empetrifolia</i>
Ag	<i>Andersonia grandiflora</i>	Du	<i>Dracophyllum uniflorum</i>	Mg	<i>Monotoca glauca</i>
Ag1	<i>Astroloma glaucescens</i>	Ec	<i>Epacris corymbifolia</i>	Ms	<i>Monotoca submutica</i>
Ah	<i>Andersonia heterophylla</i>	Ei	<i>Epacris impressa</i>	Mt	<i>Dielsiodoxa tamariscina</i>
Am	<i>Andersonia micrantha</i>	El	<i>Epacris lanuginosa</i>	Np	<i>Needhamiella pumilio</i>
Ama	<i>Astroloma macrocalyx</i>	Em	<i>Epacris marginata</i>	Pc	<i>Prionotes cerinthoides</i>
Ami	<i>Astroloma microcalyx</i>	Ep	<i>Epacris paludosa</i>	Pi	<i>Pentachondra involucreta</i>
Apa	<i>Astroloma pallidum</i>	Epe	<i>Epacris petrophilla</i>	Pp	<i>Pentachondra pumila</i>
Apr	<i>Astroloma prostratum</i>	Es	<i>Epacris serpyllifolia</i>	Ra	<i>Richea acerosa</i>
Ase	<i>Andersonia setifolia</i>	Est	<i>Epacris stuartii</i>	Rc	<i>Richea continentalis</i>
Aser	<i>Astroloma serratifolium</i>	La	<i>Leucopogon australis</i>	Rd	<i>Richea dracophylla</i>
Asp	<i>Andersonia sprengeloides</i>	Lc	<i>Leucopogon capitellatus</i>	Rm	<i>Richea milliganii</i>
Ax	<i>Astroloma xerophyllum</i>	Lci	<i>Lysinema ciliatum</i>	Rp	<i>Richea pandanifolia</i>
Bd	<i>Brachyloma daphnoides</i>	Lco	<i>Leucopogon collinus</i>	Rpr	<i>Richea procera</i>
Be	<i>Brachyloma ericoides</i>	Ld	<i>Leptecophylla divaricata</i>	Rs	<i>Richea scoparia</i>
Bp	<i>Brachyloma preissii</i>	Le	<i>Leucopogon ericoides</i>	Rsp	<i>Richea sprengeloides</i>
Cd	<i>Conostephium drummondii</i>	Lel	<i>Lysinema elegans</i>	Sh	<i>Styphelia hainesii</i>
Cg	<i>Cyathodes glauca</i>	Lf	<i>Lysinema fimbriatum</i>	Si	<i>Spengella incarnata</i>
Cm	<i>Coleanthera myrtilloides</i>	Lij	<i>Leptecophylla juniperinajuniperina</i>	Sp	<i>Spengella propinqua</i>
Cmi	<i>Conostephium minus</i>	Ljp	<i>Leptecophylla juniperinaparvifolia</i>	St	<i>Styphelia tenuifolia</i>
Cp	<i>Conostephium pendulum</i>	Li	<i>Leucopogon lanceolatus</i>	Tc	<i>Trochocarpa cunninghamii</i>
Cr	<i>Conostephium roei</i>	Lm	<i>Leucopogon montanus</i>	Tg	<i>Trochocarpa gunnii</i>
Cru	<i>Cosmelia rubra</i>	Ln	<i>Leucopogon nutans</i>	Tt	<i>Trochocarpa thymifolia</i>

FIG. 2. Ordination of epacrids according to floral trait profiles with pollinator groups that were significant predictors of the variation between species fitted as vectors: black dot = bird pollination occurs in the pollinator profile of at least one epacrid species occupying the floral ordination space; white dot = no bird pollination.

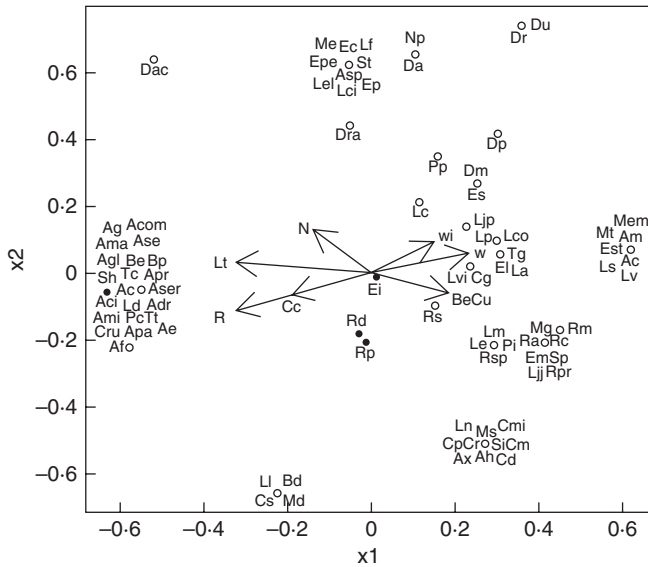


FIG. 3. Ordination of epacrids according to pollinator assemblages with floral attributes that were significant predictors of the variation between species fitted as vectors. R = red flower; Cc = constricted corolla; Lt = long floral tube; N = nectar; Wi = wide corolla; W = white flower; BeCu = bell-/cup-shaped flower; black dot = bird pollination occurs in the pollinator profile; white dot = no bird pollination. For key to species' codes, refer to Fig. 2.

Seven of the 15 floral attributes were related to the variation in the pollinator ordination space (Fig. 3; Appendix); from strongest to weakest correlation: red flower, $r = 0.562$, $P = 0.0001$; long floral tube, $r = 0.535$, $P = 0.0001$; white flower, $r = 0.391$, $P = 0.0008$; bell- or cup-shaped flower, $r = 0.316$, $P = 0.0127$; constricted corolla, $r = 0.330$, $P = 0.0084$; presence of nectar, $r = 0.312$, $P = 0.0114$; and wide corolla, $r = 0.286$, $P = 0.0302$. The vectors for red flower, long floral tube, constricted corolla and nectar were roughly opposed to those for white flower, bell- and cup-shape flower and wide corolla.

Correlations between floral attribute and pollinator dissimilarity matrices

The Mantel test showed a weak positive correlation between floral profiles and pollinator profiles overall ($r = 0.182$, $P = 0.0001$). Floral attributes were correlated with only two of the seven pollinators: bird ($r = 0.288$, $P = 0.0001$) and fly ($r = 0.079$, $P = 0.0058$) (see Appendix). Floral attributes and bird pollination both show a significant relationship and have a stronger correlation (higher r -value) than that of floral attributes and overall pollinator profiles. However, even though floral attributes and fly pollination show a statistically significant relationship, the very low r -value suggests that the relationship is very weak. There was no correlation between floral profiles and bee, beetle, butterfly, moth and wasp visitors.

Identifying pollination syndromes

Unlike most traditional statistical methods, when using the RF classifier the importance of a variable is due to its complex interactions with other variables (Liaw and Wiener, 2002; Strobl *et al.*, 2009). However, some variables have a greater impact on model success (Table 1). The model for bird-pollinated epacrids had one

TABLE 1. Random Forests classification: the mean decrease in pollination model accuracy if individual floral attributes are removed

Floral attribute	% Mean decrease in model accuracy (mean decrease in the Gini Index) for pollination model		
	Bird	Fly	Bee
Blue/purple	0.872	0.663	1.447
Red	+7.396	2.175	1.880
Pink	-1.699	1.681	1.322
White	-3.861	0.838	1.584
Green	0.227	0.474	0.476
Yellow	0.443	0.596	0.309
Brush	0.920	+2.649	+1.955
Long tube	+3.735	-3.740	-2.387
Medium tube	0.984	-2.184	1.258
Short tube	1.261	1.086	-1.962
Bell/cup	0.834	1.176	+2.642
Constricted	1.202	-3.558	1.128
Narrow	0.644	1.063	0.897
Wide	1.212	1.819	1.144
Nectar	0.284	0.476	1.202

Bold type is used for the top four predictor variables; +/- is the direction of the relationship of these variables.

Matrix

Known	RF predicted					
	Bird		Fly		Bee	
	N	Y	N	Y	N	Y
N	61	1	41	12	42	10
Y	11	14	7	27	17	18
% Out of bag estimate of error rate	13.79		21.84		31.03	

RF predicted = outcomes based on the randomly permuted out of bag (OOB) data (columns); Known = original OOB data (rows); Y = pollinator type present; N = pollinator-type absent.

floral attribute – red flower – with a relatively high impact on model success (model accuracy would undergo a mean decrease of 7.4 % if the red flower attribute was removed) and two with a moderate impact on model success – long floral tube and white flower (Table 1; Supplementary Data Fig. S1). While the presence of a red flower and long floral tube were predicative of bird pollination, white flower was predictive by its absence (i.e. its absence was more indicative of bird pollination than its presence). The bird matrix indicates that RF was unable to classify 12 of the 87 epacrids (Table 1). Random Forests classified 86.21 % of epacrids as bird or not bird pollinated (i.e. the OOB estimate of error rate or inability to classify was 13.79 %). This represented 80 % of epacrids known to be bird visited being classified as such. The model was unable to classify five of the known bird-pollinated epacrids (Supplementary Data Table S5). Modifying the data set to exclude epacrids with mixed bird and insect pollination systems had little impact on classification (Supplementary Data Table S6).

A long floral tube and constricted corolla had the greatest effect on model accuracy for fly-pollinated epacrids. Both were

negative attributes (more predictive of fly-pollinated epacrids by their absence), decreasing mean model accuracy by 3.7 and 3.6 %, respectively, if removed. The fly model matrix indicates that 19 of the 87 epacrids were unable to be classified as fly or not fly pollinated. Random Forests classified 78 % of epacrids as fly or not fly pollinated. This represented 83 % of epacrids known to be fly visited being classified as such. The model erroneously classified 11 epacrids not known to have flies as pollinators as fly pollinated.

The relative ranking of floral attribute importance for predicting bee-pollinated epacrids showed little variability, with bell- and cup-shaped flowers having the most effect on model accuracy. All attributes had an impact of between 0.3 and 2.6 on mean model accuracy. Random Forests classified 69 % of epacrids as bee or not bee pollinated, but was unable to place 27 of the 87 epacrids into either group. This represents 74 % of epacrids known to be bee visited being classified as such. In addition, the bee model wrongly classified four species not known to have bee pollination as bee pollinated.

Modifying the data set to exclude epacrids with mixed fly and bee pollination systems increased the ability of RF to classify epacrids as either fly/not fly (from 78 to 83 %) or bee/not bee pollinated (from 69 to 83 %) (Supplementary Data Table S6). For flies, short floral tubes moved from an importance ranking of nine to being the most important floral feature for model accuracy. However, no individual floral feature had a particularly great effect on model accuracy. For bees, blue and purple floral tubes moved from an importance ranking of seven to being the most important floral feature for model accuracy, although, as for fly pollination, no individual floral feature had a particularly great effect on model accuracy. There was notably less overlap in the importance ranking of floral features in the fly and bee pollination models than was apparent when they were created from the full data set.

DISCUSSION

An ornithophily pollination syndrome can be recognized in the Australian epacrids, while pollination syndromes relating to different insect groups may be less distinct (Wilson *et al.*, 2004). In general, bird pollination is known to involve tubular flowers which are often red with long floral tubes, and contain large volumes of nectar (Faegri and van der Pijl, 1979; Meléndez-Ackerman *et al.*, 1997; Schemske and Bradshaw, 1999; Wilson *et al.*, 2004; Martén-Rodríguez *et al.*, 2009; Johnson *et al.*, 2010). Fitting the floral attributes to the pollinator ordination revealed such a bird pollination syndrome in epacrids. Red flowers and long floral tubes also ranked higher than most other floral attributes as positive predictors of bird pollination in the RF classification. This result lends support to the traditional bird pollination syndrome (Faegri and van der Pijl, 1979). Thus, *Prionotes*, *Cosmelia* and many *Astroloma* flowers conform to the bird-pollinated epacrid template and are well known for this association (Paton and Ford, 1977; Ford *et al.*, 1979; Keighery, 1996; Wheal, 1996; Johnson *et al.*, 2010). Multivariate analyses on *Penstemon* (Wilson *et al.*, 2004) and Gesneriaceae (Martén-Rodríguez *et al.*, 2009) also readily identified a bird pollination syndrome where red flowers were indicative.

Conversely, the ordination of floral attributes is indicative of a generalized flora, and fitting the pollinators to it provides less

indication of an association between floral attributes and pollinator type. This suggests that while red epacrid flowers with long floral tubes are likely to be visited by birds, birds do not restrict themselves only to visiting these flowers. They also visit the red but short- or medium-tubed flowers of *Trochocarpa* and *Andersonia* (Keighery, 1996). Honeyeaters act as pollen vectors for the brush-like flowers of *Richea* (Johnson, 2012) and the smaller and lighter coloured flowers of some *Brachyloma* and *Leptecophylla* (Higham and McQuillan, 2000). Similarly, hummingbirds are mostly associated with red flowers, but they also pollinate the small, white, urceolate ericad, *Disterigma stereophyllum* (Navarro *et al.*, 2008). In New Zealand, honeyeaters are known to feed at some small apparently entomophilous flowers in genera as diverse as *Pittosporum*, *Pseudopanax*, *Dysoxylum* and *Geniostemon* (Castro and Robertson, 1997). If birds forage at a wide array of epacrid flowers, perhaps the relationship between birds and red flowers is due, at least partly, to low insect visitation to red flowers. Red flowers are difficult for bees to observe, and this may decrease the probability of bee visitation (Cronk and Ojeda, 2008).

It may be expected that broad foraging by birds, plus the bimodality of some epacrid pollination systems where birds and insects forage at the same plants, are likely to make the syndrome less reliable. Thus, the RF model for bird pollination in the Australian epacrids was able to classify 86 % rather than 100 % of the species studied as bird pollinated or not bird pollinated. A classification of 100 % is unlikely to be achieved as the RF pollination model was built by distilling the typical floral features correlated with birds in the data set provided. The model does not fit epacrids where the typical morphological adaptations are absent. For instance, RF was unable to recognize as bird pollinated the white, long-tubed *Astroloma macrocalyx* and *Leptecophylla divaricata*, the white brush flower *Richea dracophylla*, and the pink and short-tubed *Trochocarpa cunninghamii* and *Brachyloma ericoides*. Currently, *R. dracophylla* is the only one of these known to have a mixed bird and insect pollination system. Perhaps the longer floral tubes of *L. divaricata* and *A. macrocalyx* prevent insect access to the nectar. The bird-pollinated epacrid, *Prionotes cerinthoides*, is accessed by honeyeaters with relatively long beaks, but the tube length prevents insects from reaching the nectar held at the base of the tube (unless they resort to nectar-robbing by piercing the corolla at its base) (Johnson *et al.*, 2010). Similarly, the hairy-covered corolla mouth of *T. cunninghamii* may act to exclude insect access. *Trochocarpa* includes taxa (such as *T. cunninghamii*) with red and hairy flowers visited by birds, and taxa (such as *T. gunnii*) with white open cup-shaped and hairless flowers visited by insects.

Bees and flies forage on many of the same epacrids, as indicated by the close proximity of their vectors on the floral ordination. In general, they appear to be important pollinators of brush, short-tubed and bell/cup-shaped epacrids (epacrids lacking long floral tubes). Bees and flies also foraged at the bird-visited flowers of *Epacris impressa* and *Richea dracophylla*. Morphological variability is apparent within *E. impressa* which has longer corollas in its pink- and red-flowered races and shorter corollas in its white-flowered race, exhibiting a bias towards bird and insect pollination, respectively (Stace and Frupp, 1977; Hingston and McQuillan, 2000). Thus, there may

be less of a foraging overlap on any individual *E. impressa* plant than the results indicate. In contrast, the brush flowers of *Richea* are highly generalized and open to all visitors. In a global test of the utility of the traditional pollination syndromes, Ollerton *et al.* (2009) found that bee- and fly-pollinated plants were accurately predicted more frequently than other syndromes. Yet, in epacrids there is generally only a weak relationship between floral attributes and flies or bees; and bird pollination tends to be more accurately predicted. The lack of relatively high ranking positive floral attributes in the classification defining fly and bee pollination in epacrids (such as the red flower and long floral tube for bird pollination) suggests that there is no obvious specialization of the individual floral features used in the study, and thus no obvious pollination syndrome (other than perhaps a generalist one).

Bees are perhaps the insects best suited for the process of pollination (Faegri and van de Pijl, 1979). The RF classification suggests bees are generalized foragers on epacrids – no individual floral attribute assumed a high level of importance in model accuracy. While bees forage at allophilic flowers, they also have access to euphilic flowers exhibiting a particular syndrome (Faegri and van der Pijl, 1979). In the epacrids, they appear to fill a generalist role plus the niche-role of buzz-pollinating the nectarless flowers of *Sprengelia incarnata* and *Conostephium pendulum* (Houston and Ladd, 2002; Johnson and McQuillan, 2011) and potentially *Coleanthera* and *Rupicola* (Houston and Ladd, 2002). The Styphelioideae lack the zygomorphic, bright yellow flowers or flowers with nectar guides that typify the traditional bee pollination syndrome (Faegri and van der Pijl, 1979). However, blue flowers also form part of the bee pollination syndrome (Faegri and van der Pijl 1979), and four of the six blue or purple flowers in the data set were pollinated by bees and none by flies. When the RF floral data set was modified to exclude epacrids pollinated by both bees and flies, this difference became apparent in the RF floral classification model, which showed blue and purple flowers to have the greatest impact on bee pollination model accuracy.

Flies are important pollinators in cooler environments, such as southern Australia, where they are present all year and cover a greater altitudinal range than bees (Hingston and McQuillan, 2000). However, the correlation between fly pollination and floral features was tenuous ($r = 0.079$). Similarly, the highest ranking predictors of fly pollination in the RF classification were a long floral tube and constricted corolla, which were only predictive by their absences. When the RF floral data set was modified to exclude epacrids pollinated by both bees and flies, the presence of short floral tubes became the most important floral feature. Unlike the bird-visited flowers, colour was not a strong predictor variable, with the absence of red flowers being the first reference to floral colour in the predictor profile. The generalist pollination syndrome exhibited by epacrids visited by flies is consistent with that described by Faegri and van der Pijl (1979) which observed that flies were generally restricted to dull-coloured, actinomorphic flowers with exposed rewards.

In conclusion, while most epacrids have generalized pollination systems, some evidence of a bird pollination syndrome is found. Birds visit a wide variety of flowers, including many that are not red and do not have long floral tubes; thus, the syndrome only partially captures the extent of bird pollination in the epacrids. This may partially relate to bird and insect vision, with red flowers standing out from their surroundings for birds

but being hidden from some insects. While long floral tubes are not necessary for birds to visit flowers, they may exclude insects with short proboscises from foraging on nectar held at the base of long tubes. Thus, this combination of traits may equate to a bird pollination syndrome not only because they reflect some of the flower types that birds forage on, but because they also limit insect foraging.

In the case of the Australian epacrids, the presence of mixed insect pollination systems makes classification of individual pollination systems less reliable. Removing epacrids with mixed bee and fly pollination systems from the analyses improved the classification rate of both pollination systems. However, it did not expose any particularly important floral traits for classifying each syndrome, reflecting the generalized nature of the insect-pollinated epacrids, at least where flower type, colour, corolla length and width, and nectar presence were concerned. In future analyses, the inclusion of nectar composition and scent may assist in distinguishing between different insect pollinators and revealing greater specialization in the epacrid flora than is currently apparent (Faegri and van der Pijl, 1979; Huber *et al.*, 2005; Chess *et al.*, 2008).

Random Forests provides insight into the differences between the contribution of each floral attribute and the relative importance in relation to a particular pollinator, thus highlighting the major phenotypic convergences. In the Australian epacrid flora, red flowers and long floral tubes are ranked positively as floral predictors for birds and negatively as predictors for both bees and flies. For flies, the easy access given by open flowers or those with short floral tubes assumes greater importance than any particular colour. Thus, each floral trait assists either positively or negatively in defining a pollination syndrome, and tells us something of the foraging preferences of different pollinator types. The limitations of this approach (along with the availability of information on both pollination systems and floral attributes particularly regarding the reproductive organs, nectar composition and quantity, and flower scent) include our limited data set and our limited understanding of which traits attract pollinators, and how to interpret them in a way that reflects pollinator cognition (Ollerton *et al.*, 2009). The predictive value of the RF analysis performed here is necessarily specific to the Australian epacrids upon which the classification was performed. Given a full data set of epacrid floral traits and pollinators, RF analysis could vastly increase our knowledge of which traits attract pollinators and potentially tease out more of the differences between the insect groups. Overall, RF provides a wholistic approach for exploring pollination syndromes and is worthy of further use in pollination ecology. With the recent publication of a genus-level epacrid phylogeny (Johnson *et al.*, 2012), the next step is to explore epacrid pollination systems in a phylogenetic context to pin down the evolutionary shifts between pollinator types, particularly bird versus insect.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: field observations: site summary. Table S2: potential pollinators of epacrids, sorted by tribe, plant species, common name of potential pollinator. Table S3: flora trait profiles for epacrid species. Table S4: potential pollinator profiles for epacrid species.

Table S5: predicted pollinators: Random Forests. Table S6: modified data sets and RF model accuracy. Figure S1: percentage mean decrease in model accuracy if selected floral attributes are removed, and details of the four most important floral predictor variables. Figure S2: percentage mean decrease in RF model accuracy if selected floral attributes are removed for modified data sets.

ACKNOWLEDGEMENTS

I am grateful to B. Holland and S. Wotherspoon (UTAS) for technical expertise and useful suggestions regarding the Random Forests analyses; and J. Kirkpatrick and B. Holland for comments on an early manuscript draft. Comments from A. Hingston, J. Ollerton and an anonymous reviewer improved the manuscript.

LITERATURE CITED

- Allan, HH. 1961. *Flora of New Zealand*. Wellington: Government Printer.
- Andersson S, Nilsson LA, Groth I, Bergstrom G. 2002. Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. *Botanical Journal of the Linnean Society* **140**: 129–153.
- Armesto JJ, Smith-Ramirez C, Sabag C. 1996. The importance of plant–bird mutualisms in the temperate rainforest of southern South America. In: Lawford RG, Alaback PB, Fuentes E. eds. *High-latitude rainforests and associated ecosystems of the west coast of the Americas*. New York: Springer-Verlag, 248–265.
- Breiman L. 2001. Random forests. *Machine Learning* **45**: 5–32.
- Brown EA, Streiber N. 1999. Systematic studies in *Dracophyllum* (Epacridaceae) 2. New species of *Dracophyllum* in New South Wales. *Telopea* **8**: 393–401.
- Castellanos MC, Wilson P, Thomson JD. 2004. ‘Anti-bee’ and ‘pro-bird’ changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Evolutionary Biology* **17**: 876–885.
- Castellanos MC, Wilson P, Keller SJ, Wolfe AD, Thomson JD. 2006. Anther evolution: pollen presentation strategies when pollinators differ. *American Naturalist* **167**: 288–296.
- Castro I, Robertson AW. 1997. Honeyeaters and the New Zealand forest flora: the utilisation and profitability of small flowers. *New Zealand Journal of Ecology* **21**: 169–179.
- Celebrezze T, Paton DC. 2004. Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). *Austral Ecology* **29**: 129–136.
- Chess SKR, Raguso RA, LeBuhn G. 2008. Geographic divergence in floral morphology and scent in *Linanthus dichotomus* (Polemoniaceae). *American Journal of Botany* **95**: 1652–1659.
- Corbett C. 1995. *Pollination ecology in a Tasmanian alpine environment*. Honours thesis. University of Tasmania, Hobart, Australia.
- Corrick MG, Fuhrer BA, George AS. 1996. *Wildflowers of Western Australia*. Melbourne, Australia: The Five Mile Press Ltd in association with Monash University.
- Cronk Q, Ojeda I. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* **59**: 715–727.
- Curtis WM. 1963. *The student's flora of Tasmania*. Hobart, Australia: Government Printer.
- Cutler DR, Edwards TC, Beard KH, et al. 2007. Random forests for classification ecology. *Ecology* **88**: 2783–2792.
- Doull K. 1973. Bees and their role in pollination. *Australian Plants* **7**: 223, 230–231.
- Faegri K, van de Pijl L. 1979. *The principles of pollination ecology*, 3rd edn. Oxford: Pergamon Press.
- Fenster CB, Armbruster PW, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* **35**: 375–403.
- Fletcher H. 1977. *Habitat relationships among small mammals at Petroi, north-eastern New South Wales*. Masters thesis. University of New England, Armidale.
- Ford HA, Paton DC, Forde N. 1979. Birds as pollinators of Australian plants. *New Zealand Journal of Botany* **17**: 509–519.
- Goldblatt P, Manning JC. 2005. Radiation of pollination systems in the Iridaceae of sub-saharan Africa. *Annals of Botany* **97**: 317–344.
- Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* **22**.
- Green K, Osborne W. 1994. *Wildlife of the Australian snow-country: a comprehensive guide to alpine fauna*. Chatswood, New South Wales, Australia: Reed Books.
- Harden GJ. 1992. *Flora of New South Wales*. Kensington: New South Wales University Press.
- Higham RK, McQuillan PB. 2000. *Cyathodes divaricata* (Epacridaceae) – the first record of a bird pollinated dioecious plant in the Australian flora. *Australian Journal of Botany* **48**: 93–99.
- Hingston AB, McQuillan PB. 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania? *Austral Ecology* **25**: 600–609.
- Houston TF. 2000. *Native bees on wildflowers in Western Australia: a synopsis of bee visitation of wildflowers based on the bee collection of the Western Australian Museum, Special Publication No. 2*. Perth: Western Australian Insect Study Society.
- Houston TF, Ladd PG. 2002. Buzz pollination in the Epacridaceae. *Australian Journal of Botany* **50**: 83–91.
- Huber FK, Kaiser R, Sauter W, Schiestl FP. 2005. Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). *Oecologia* **142**: 564–575.
- Jessop JP, Toelken HR. 1986. *Flora of South Australia*. Adelaide: South Australian Printing Division.
- Johnson KA. 2012. *Pollination ecology and evolution of epacrids*. PhD thesis. University of Tasmania, Hobart, Australia.
- Johnson KA, Holland BR, Heslewood MM, Crayn DM. 2012. Supermatrices, supertrees and serendipitous scaffolding: inferring a well-resolved genus-level phylogeny of Styphelioideae (Ericaceae) despite missing data. *Molecular Phylogenetics and Evolution* **62**: 146–155.
- Johnson KA, McQuillan PB. 2011. Comparative floral presentation and bee-pollination in two *Sprengelia* species (Ericaceae). *Cunninghamia* **12**: 45–51.
- Johnson KA, McQuillan PB, Kirkpatrick JB. 2010. Bird pollination of the climbing heath *Prionotes cerinthoides* (Ericaceae). *International Journal of Plant Sciences* **171**: 147–157.
- Johnson KA, McQuillan PB, Kirkpatrick JB. 2011. Nocturnal mammals, diurnal lizards and the pollination ecology of the cryptic flowering *Acrotriche serrulata* (Ericaceae). *International Journal of Plant Sciences* **172**: 173–182.
- Kay KM, Schemske DW. 2003. Pollinator assemblages and visitation rates for 11 species of neotropical *Costus* (Costaceae). *Biotropica* **35**: 198–207.
- Keighery GJ. 1996. Phytogeography, biology and conservation of Western Australian Epacridaceae. *Annals of Botany* **77**: 347–356.
- Laing RM, Blackwell EW. 1964. *Plants of New Zealand*. Christchurch: Whitcombe and Tombs Ltd.
- Liaw A, Wiener M. 2002. Classification and regression by randomforest. *RNews* **2**: 18–22.
- Mark AF, Adams NM. 1986. *New Zealand alpine plants*. Auckland, New Zealand: Reed Meuthen.
- Martén-Rodríguez S, Almarales-Castro A, Fenster CB. 2009. Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers. *Journal of Ecology* **97**: 348–359.
- Meléndez-Ackerman E, Campbell DR, Waser NM. 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology* **78**: 2532–2541.
- Navarro L, Guitián P, Ayensa G. 2008. Pollination ecology of *Disterigma stereophyllum* (Ericaceae) in south-western Colombia. *Plant Biology* **10**: 512–518.
- Newstrom L, Robertson AW. 2005. Progress in understanding pollination systems in New Zealand. *New Zealand Journal of Botany* **43**: 1–59.
- Ollerton J, Johnson SD, Cranmer L, Kellie S. 2003. The pollination ecology of an assemblage of grassland asclepiads in South Africa. *Annals of Botany* **92**: 807–834.
- Ollerton J, Alarcon R, Waser NM, et al. 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* **103**: 1471–1480.
- Olsson M, Shine R, Ba’k-Olsson E. 2000. Lizards as a plant’s ‘hired help’: letting pollinators in and seeds out. *Linnean Society of London* **71**: 191–202.
- Packowska G, Chapman AR. 2000. *The Western Australian flora: a descriptive catalogue*. Wildflower Society of Western Australia (Inc.), the Western

Australian Herbarium, CALM, and the Botanic Gardens and Parks Authority, Perth.

- Paton DC, Ford HA. 1977.** Pollination by birds of native plants in South Australia. *Emu* **77**: 73–85.
- Perez F, Arroyo MTK, Medel R, Hershkovitz MA. 2006.** Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *American Journal of Botany* **93**: 1029–1038.
- Schemske D, Bradshaw HD. 1999.** Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences, USA* **96**: 11910–11915.
- Schneemilch M, Williams C, Kokkinn M. 2011.** Floral visitation in the Australian native shrub genus *Acrotriche* R.Br (Ericaceae): an abundance of ants (Formicidae). *Australian Journal of Entomology* **50**: 130–138.
- Semmens TD. 1993.** *Bombus terrestris* (L.) (Hymenoptera: Apidae) now established in Tasmania. *Journal of the Australian Entomological Society* **32**: 346.
- Stace HM, Fripp YJ. 1977.** Racialization in *Epacris impressa*. II. Habitat differences and flowering times. *Australian Journal of Botany* **25**: 315–323.
- Stephens PF, Luteyn J, Oliver EGH, et al. 2004.** Ericaceae. In: Kubitzki K. ed. *The families and genera of vascular plants*, Vol. 6. Berlin: Springer-Verlag, 145–194.
- Strobl C, Boulestreix A, Kneib T, Augustin T, Zeileis A. 2008.** Conditional variable importance for random forests. *BMC Bioinformatics* **9**: 307.
- Strobl C, Malley J, Tutz G. 2009.** An introduction to recursive partitioning: rationale, application, and characteristics of classification and regression trees, bagging and random forests. *Psychological Methods* **14**: 323–348.
- Thomson JD, Wilson P, Valenzuela M, Malzone M. 2000.** Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology* **15**: 11–29.
- Van Royen P. 1982.** *The alpine flora of New Guinea*. Hirschberg: Strauss and Cramer.
- Walsh NG, Entwistle TJ. eds. 1996.** Flora of Victoria: dicotyledons (Winteraceae to Myrtaceae), 3. Inkata Press.
- Wheal M. 1996.** Movement patterns of honeyeaters foraging alone and in flocks for nectar of *Astroloma conostephioides* in Hale Conservation Park, South Australia. *Emu* **96**: 55–61.
- Wheeler J, Marchant N, Lewington M. 2002.** *Flora of the south west: Bunbury-Augusta-Denmark*. Perth: Western Australian Herbarium.
- Whittall JB, Hodges SA. 2007.** Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**: 706–709.
- Wilson P, Castellanos MC, Hogue JN, Thomson JD, Armbruster WS. 2004.** A multivariate search for pollination syndromes among penstemons. *Oikos* **104**: 345–361.

Pollinator attributes in floral ordination space

	1	2	<i>r</i>	<i>P</i> -value
Fly	–0.9999	–0.0111	0.4161	0.0005
Bee	–0.8572	0.5150	0.4453	0.0001
Beetle	–0.2610	–0.9653	0.1951	0.1967
Butterfly	–0.4479	–0.8941	0.2298	0.1043
Wasp	–0.8319	0.5549	0.1562	0.3623
Bird	0.9668	0.2554	0.5274	0.0001
Moth	0.1579	–0.9875	0.2027	0.1769

Significant values are highlighted in bold.

Mantel tests: floral traits (Euclidean distance matrix) correlated with Bray–Curtis distance matrices for all pollinator types and individual pollinator types below

Bray–Curtis matrix				Lower limit	Upper limit	
	mantelr	<i>P</i> -value1	<i>P</i> -value2	2.5 %	97.5 %	
Pollinator	0.1820	0.0001	1.0000	0.0001	0.1423	0.2217
Bird	0.2878	0.0001	1.0000	0.0001	0.2461	0.3368
Fly	0.0788	0.0058	0.9943	0.0058	0.0516	0.1104
Bee	0.0398	0.0515	0.9486	0.0535	0.0176	0.0639
Beetle	–0.0526	0.8815	0.1186	0.2503	–0.0755	–0.0234
Moth	–0.0583	0.8497	0.1504	0.3148	–0.0910	–0.0210
Wasp	–0.0598	0.8433	0.1568	0.3109	–0.1041	–0.0183
Butterfly	–0.0605	0.9848	0.0153	0.0740	–0.0822	–0.0384

Significant values are highlighted in bold.

APPENDIX

Floral attributes in pollinator ordination space

	1	2	<i>r</i>	<i>P</i> -value
Blue/purple	0.1882	–0.9821	0.1876	0.2255
Red	–0.9446	–0.3282	0.5620	0.0001
Pink	–0.4967	–0.8679	0.1480	0.3935
White	0.9686	0.2485	0.3909	0.0008
Green	–0.6414	0.7672	0.0054	0.9978
Yellow	0.8681	–0.4964	0.1225	0.5488
Brush	0.5702	–0.8215	0.2060	0.1577
Long-tubed	–0.9950	0.1004	0.5347	0.0001
Medium-tubed	–0.0721	0.9974	0.1424	0.4205
Short-tubed	0.9245	0.3812	0.2112	0.1480
Bell-/cup-shaped	0.9473	–0.3202	0.3159	0.0127
Constricted	–0.9498	–0.3128	0.3296	0.0084
Narrow	–0.9539	0.3002	0.1690	0.2981
Wide	0.8479	0.5301	0.2864	0.0302
Nectar	–0.7323	0.6810	0.3119	0.0114

Significant values are highlighted in bold.