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Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation

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Summary

1. The relative importance of specialized and generalized plant-pollinator relationships is contentious, yet analyses usually avoid direct measures of pollinator quality (effectiveness), citing difficulties in collecting such data in the field and so relying on visitation data alone.

2. We demonstrate that single-visit deposition (SVD) of pollen on virgin stigmas is a practical measure of pollinator effectiveness, using 13 temperate and tropical plant species. For each flower the most effective pollinator measured from SVD was as predicted from its pollination syndrome based on traditional advertisement and reward traits. Overall, *c.* 40% of visitors were not effective pollinators (range 0–78% for different flowers); thus, flower–pollinator relationships are substantially more specialized than visitation alone can reveal.

3. Analyses at species level are crucial, as significant variation in SVD occurred within both higher-level taxonomic groups (genus, family) and within functional groups.

4. Other measures sometimes used to distinguish visitors from pollinators (visit duration, frequency, or feeding behaviour in flowers) did not prove to be suitable proxies.

5. Distinguishing between ‘pollinators’ and ‘visitors’ is therefore crucial, and true ‘pollination networks’ should include SVD to reveal pollinator effectiveness (PE). Generating such networks, now underway, could avoid potential misinterpretations of the conservation values of flower visitors, and of possible extinction threats as modelled in existing networks.

Key-words: flower visitor, network, pollen deposition, pollination syndromes, pollinator, specialization/generalization

Introduction

Pollination ecology has recently been invigorated by a strong community-level approach, often linked with concern over pollinator declines and conservation, and hence a need to understand how particular pollinator deficits may affect plant and animal populations and interactions (Waser *et al.* 1996). This has led to many analyses of ‘pollination webs’ or ‘pollination networks’, aiming to understand network structure and resilience to change. As networks have become embedded in ecological and evolutionary thinking (Proulx, Promislow & Phillips 2005), ‘plant–pollinator networks’ proliferate and associated methodologies and terminologies become more sophisticated. Core problems of inadequacy of the underlying data sets (incomplete sampling or varied relative sampling intensity, Blüthgen *et al.* 2008; Gibson *et al.* 2011), and of inadequate temporal/spatial spread of sampling (Olesen *et al.* 2008; Dupont *et al.* 2009) have been addressed. The resultant more complex models are often in turn used in meta-analyses: comparisons with other mutualistic communities (Olesen *et al.* 2007; Aizen, Morales & Morales 2008; Pocock, Evans & Memmott 2012), or assessing effects of invasive species (Memmott & Waser 2002; Bartomeus, Vila & Santamaria 2008; Valdovinos *et al.* 2009), of potential extinction rates and patterns (Memmott, Waser & Price 2004; Kaiser-Bunbury *et al.* 2010), or of resilience to anthropogenic factors such as climate change (Memmott *et al.* 2007; Willmer 2012).

This modelling activity has become linked with issues of specialization and generalization in plants, pollinating animals and their interactions (Waser *et al.* 1996; Johnson & Steiner 2000; Gibson *et al.* 2011). ‘Plant–pollinator networks’ appear to have flower visitors that are mostly generalized in their flower choices (Vazquez & Aizen 2004; Petanidou & Potts 2006), in turn suggesting that the concept of specific ‘pollination syndromes’ is less useful than the earlier literature had indicated (Waser 2006; Ollerton *et al.* 2009).

These issues have been highlighted in several key papers (Waser *et al.* 1996; Fenster *et al.* 2004) and a recent book (Willmer 2011), though the network and syndrome approaches can potentially be synergistic. Many ‘pollinator networks’ suggest preponderant generalization with high connectance, but merely eliminating cheats can make a network register as more specialized (Alarcón 2010), and levels of apparent

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generalization can vary across populations or even individuals of a given plant species (Herrera 2005). Meanwhile many pollination case studies report rather high levels of specialization, and/or a good match of selective pressures on flowers to particular functional groups of visitors acting as pollinators (Johnson & Steiner 2000; Fenster *et al.* 2004).

But a key issue still goes largely untested: the ability to distinguish between mere flower visitors and effective pollinators. This problem is well documented (Fishbein & Venable 1996; Ne'eman *et al.* 2010; Popic, Wardle & Davila 2013), and many 'pollination networks' explicitly or implicitly recognize the potentially misleading title used, in relying on simple visitation records. But variations on the claim that 'pollination can be inferred if quantitative data is available on visitation' (Hegland *et al.* 2010) remain prevalent.

Testing this requires incorporation of measures of effective pollination into community studies and thence into networks. Some studies add a more realistic 'pollination' slant to visitation data using various added measures (reviewed in Ne'eman *et al.* 2010; most recently using visitor pollen loads, Popic, Wardle & Davila 2013), but as yet sidestep measuring effectiveness of visitors as true pollinators. Here, we quantify the 'pollinator vs. visitor' problem to show that the distinction matters greatly and may undermine some existing literature. We use the term *pollinator effectiveness* (PE) throughout, rather than other variant terms (Inouye *et al.* 1994; Ne'eman *et al.* 2010), agreeing that it best describes the character of the measure needed. Ne'eman and his co-authors supported (from first principles) measuring numbers of conspecific pollen grains deposited on a virgin stigma in one visit – single-visit deposition, hereafter SVD. This measures both an animal's ability to acquire pollen in earlier visits to the plant species (thus incorporating visit constancy), and to accurately deposit it where it can potentially lead to fertilization. It avoids hazards of measurements of seed- or fruit-set that bring postpollination factors into play, and it gives species-specific values for PE. It can be expanded to give SVD per unit time (hour, or day), or through the life of the flower, or plant, or population. Some early papers had shown that this field measurement could indeed clarify the visitor–pollinator distinction. Good models of best practice exist (Primack & Silander 1975; Motten *et al.* 1981; Wilson & Thomson 1991), and examples occur for bees, flies, lepidopterans and vertebrates (Willmer 2011).

Our field measurements demonstrate that pollinator effectiveness (PE) is reliably and relatively easily determined using SVD, for 13 plant species from various traditional 'syndromes'. True pollination networks are therefore feasible and much needed, and this ongoing work will improve understanding of the pressing issues of pollination ecosystem services and pollinator conservation.

Materials and methods

PLANTS AND STUDY SITES

We used 13 plant species (Table S1), from two temperate Scottish sites (scrubby woodland, West Quarry Braes, Fife (NO 597 088) and meadowland near Loch Tay, [NN 669 358)], and from deciduous forest in Costa Rica (Santa Rosa, 10°50′N, 85°40′W). Plants were selected for their flowers' apparent conformity to particular pollination syndromes with a broad range of morphological and reward traits.

MEASURING POLLINATOR EFFECTIVENESS

Flowers were selected as buds, usually in the evening, and covered (individually, in small groups, or as inflorescences) in 2 mm netting to exclude flower visitors but avoid excessive environmental modification. Once flowers had fully opened the next day they were uncovered and observed until a single visitor landed and foraged. Visitors were identified immediately, or photographed, or captured for later identification. Each visit's duration was timed using a stopwatch, or by estimation (to nearest 10 or 30 s) where a visitor fed successively at several flowers on an inflorescence (mean duration shown without SE in Table 1), or where several visitors were active concurrently. For hummingbirds, hovering between flower visits, durations were corrected to give mean time spent feeding using video recordings. Visitor feeding (nectar, pollen or both) was also recorded.

Stigmas from each visited flower (or each floret visited in a composite) were then removed with forceps and stored in separate cells of plastic cell-culture arrays, kept covered and cool. Numbers of adherent pollen grains per stigma were counted immediately using a dissecting microscope; or the array was stored frozen for later counting. Pollen grains were only counted if morphologically conspecific.

For each plant species, unvisited flowers were also netted as controls, and pollen grains on their stigmas recorded to account for self-pollen transfer by wind or by flower handling. A value of mean SVD was determined for each visitor species for which sufficient data were available, and compared to the control SVD. A pollinator was defined as *any species with an SVD significantly greater than controls*. All other visitor species were deemed ineffective pollinators (including, but not synonymous with, floral thieves) and excluded from further analysis.

SAMPLING PERIODS

Sampling occurred throughout a day where possible, to detect temporal variations in visitor assemblage and performance. Observations were restricted to dry calm weather conditions, when previously protected flowers were unaffected by rain. Sampling sessions were 1–3 h, depending on visit frequency and thus how long it took all previously-protected newly-opened flowers to be visited.

VISITATION SURVEYS: SCALING UP SVDS AND POLLINATOR EFFECTIVENESS

Observations of flower visits necessarily only applied to the *first* visitor to previously-netted flowers, so cannot accurately represent overall visit numbers or frequencies. To record both visitation patterns and SVD separately, we chose Scottish populations of *Agrimonia eupatoria*, with large well-spaced flowers on adjacent stems. Flowers were observed for twelve 45-min intervals daily (06:45–18:30, with all flowers by then pollen-depleted) in July 2009. Visit frequencies, durations and behaviours of each visitor were recorded. Since visitors were undisturbed they visited a sequence of flowers freely, and their chosen flowers were noted. Visitors were mainly hoverflies, taking only pollen; most were identified to species (but to tribe for Bacchini and Syrphini) and a mean SVD was calculated. Combined with visitor frequencies this generated a per-hour and per-day pollinator performance value from existing formulae (Ne'eman *et al.* 2010).

Table 1. Mean single-visit deposition (SVD) values (±SE) for each visitor group, and all visitor species where $n > 5$ or P-value significant, for the 13 plant species, with significance indicated as the difference in SVD (corrected for mean pollen on unvisited control flowers, value in parentheses alongside plant name) from zero. P-value bold where $P < 0·05$ (*where significance also meets the criteria of Bonferroni's correction). Final column shows mean visit duration (±SE). Spearman's rank correlations for SVD/duration comparisons (overall, and split by visitor species) are also shown.

	Mean SVD	n	P-value	Mean visit duration (s)
Malvaviscus (10·6)				
Hummingbirds (*Amazilia rutila*)	104·4 ± 9·8	21	**< 0·0005***	6·1 ± 1·2
Bees	29·0	35	**< 0·0005***	92·1 ± 9·2
Agapostemon sp.	53·1 ± 15·3	8	**0·008**	91·9 ± 23·8
Trigona fulviventris	21·9 ± 5·5	13	**0·018**	110·8 ± 17·8
Tetragonisca angustula	21·9 ± 4·3	14	**0·008**	75·0 ± 8·2
Butterflies	5·8 ± 1·7	12	0·180	122·5 ± 21·0
Ants (*Camponotus novograndensis*)	11·1 ± 1·5	8	0·066	180·0 ± 29·9
SVD vs. duration: $r = -0·64$, $n = 76$, $P < 0·001$. Split by visitor species: NS				
Helicteres (89·0)				
Hummingbirds (*Phaethornis guy*)	1517·1 ± 97·5	21	**< 0·0005***	1·73 ± 0·4
Bees	441·8	105	**< 0·0005***	202·0 ± 10·0
Trigona fulviventris	443·4 ± 29·9	92	**< 0·0005***	232·5 ± 10·8
Agapostemon sp.	400·0 ± 101·4	6	**0·028**	80·0 ± 24·1
Tetragonisca angustula	162·9 ± 26·0	7	**0·028**	68·6 ± 14·2
SVD vs. duration: $r = -0·41$, $n = 127$, $P < 0·001$. Split by visitor species: NS				
Geranium (16·7)				
Bees	33·9	56	**< 0·0005***	23·8 ± 2·5
Bombus pratorum	31·2 ± 6·7	52	**< 0·0005***	25·2 ± 2·6
Flies	19·8	25	**0·027**	48·0 ± 7·9
Rhingia campestris	19·0 ± 5·8	19	**0·012***	42·6 ± 5·8
SVD vs. duration: $r = 0·19$, $n = 75$, $P = 0·103$. Split by visitor species: *B. pratorum* ($r = +0·32$; $P = 0·019$)				
Digitalis (19·4)				
Bees	58·2	38	**< 0·0005***	16·1 ± 1·6
Bombus hortorum	73·2 ± 16·7	25	**< 0·0005***	11·4 ± 1·3
Bombus muscorum	31·0 ± 4·4	12	**0·005***	26·3 ± 2·6
SVD vs. duration: $r = -0·15$, $n = 37$, $P = 0·362$. Split by visitor species: NS				
Byrsonima (48·5)				
Bees	313·9	82	**< 0·0005***	65·9 ± 6·0
Exomalopsis sp.	1686·7 ± 121·7	3	0·109	20·0 ± 5·8
Centris nitida	381·7 ± 96·8	6	**0·043**	45·0 ± 5·5
Trigona fulviventris	254·5 ± 29·9	61	**< 0·0005***	64·9 ± 5·3
Tetragonisca angustula	238·8 ± 41·3	12	**< 0·003***	92·5 ± 29·7
SVD vs. duration: $r = -0·14$, $n = 82$, $P = 0·202$. Split by visitor species: NS				
Agrimonia (8·5)				
Hoverflies	36·2	139	**< 0·0005***	24·1 ± 1·4
Rhingia campestris	55·2 ± 21·9	15	**0·005***	20·0
Platycheirus scutatus	52·8 ± 8·1	19	**< 0·0005***	30·0
Platycheirus albimanus	47·6 ± 19·2	10	**0·008**	63·5 ± 7·9
Leucozona laternaria	43·5 ± 10·5	12	**0·008**	20·0
Episyrphus balteatus	27·6 ± 2·9	63	**< 0·0005***	19·9 ± 1·6
Meliscaeva auricollis	23·2 ± 6·7	13	**0·012**	16·5 ± 1·3
SVD vs. duration: $r = 0·11$, $n = 141$, $P = 0·177$. Split by visitor species: NS				
Cirsium (0)				
Bees (*Bombus terrestris*)	1·8 ± 0·2	22	**0·038**	19·1 ± 2·4
Hoverflies	2·9	53	**< 0·0005***	8·8 ± 1·7
Episyrphus balteatus	3·8 ± 0·8	26	**< 0·0005***	8·7 ± 3·5
Platycheirus manicatus	2·1 ± 0·3	16	**0·002***	7·5 ± 0·5
Melanostoma mellinum	2·1 ± 0·8	11	**0·001***	10·9 ± 0·3
Other Flies	1·2	31	**< 0·0005***	20·6 ± 1·6
Empis sp.	1·8 ± 0·5	5	**< 0·0005***	36
Calliphora vomitoria	1·2 ± 0·1	15	**< 0·0005***	22·7 ± 0·8
SVD vs. duration: $r = -0·22$, $n = 106$, $P = 0·021$. Split by visitor species: *B. terrestris* ($r = +0·63$, $P = 0·001$), *M. dubium* = ($r = +0·77$, $P < 0·001$): *C. vomitoria* ($r = +0·53$, $P = 0·040$)				
Centaurea (14·0)				
Hoverflies	217·9	240	**< 0·0005***	11·4 ± 0·7
Episyrphus balteatus	273·7 ± 41·7	158	**< 0·0005***	8·2 ± 0·2
Eupeodes corollae	115·0 ± 23·6	12	**0·002***	15
Rhingia campestris	114·1 ± 13·9	65	**< 0·0005***	18·6 ± 2·4
Platycheirus manicatus	50·4 ± 25·8	5	0·109	6
SVD vs. duration: $r = -0·25$, $n = 240$, $P < 0·001$. Split by visitor species: *R. campestris* ($r = -0·60$; $P < 0·001$)				

Table 1. (continued)

	Mean SVD	*n*	*P*-value	Mean visit duration (s)
Knautia (0)				
Bees	4·9	66	**< 0·0005***	6·7 ± 0·7
Bombus pratorum	6·0 ± 0·9	21	**< 0·0005***	4·3 ± 0·8
Bombus (*Psithyrus*) *bohemicus*	5·9 ± 1·3	19	**0·001***	1·6
Bombus lucorum	4·8 ± 0·7	12	**0·002***	10·0
Bombus terrestris	2·1 ± 1·0	14	**0·018**	14·3 ± 0·5
Hoverflies	5·8	303	**< 0·0005***	3·2 ± 0·2
Rhingia campestris	7·4 ± 1·4	54	**< 0·0005***	2·2 ± 0·1
Episyrphus balteatus	6·4 ± 0·6	203	**< 0·0005***	3·6 ± 0·3
Syrphus ribesii	1·0 ± 0·2	42	**0·018**	1·8 ± 0·1
Other dipterans (*Empis* sp.)	6·1 ± 0·6	147	**< 0·0005***	7·9 ± 0·5
SVD vs. duration: $r = -0{\cdot}11$, $n = 516$, $P = 0{\cdot}016$. Split by visitor species: *R. campestris* ($r = +0{\cdot}64$; $P < 0{\cdot}001$), *E. balteatus* ($r = -0{\cdot}41$; $P < 0{\cdot}001$)				
Trifolium (0·6)				
Bees	12·2	371	**< 0·0005***	3·2 ± 0·1
Bombus lucorum	25·1 ± 2·2	31	**< 0·0005***	1·3 ± 0·1
Bombus terrestris	13·3 ± 1·5	34	**< 0·0005***	1·5 ± 0·1
Bombus hortorum	10·8 ± 0·6	275	**< 0·0005***	3·7 ± 0·1
Bombus muscorum	10·0 ± 1·8	31	**< 0·0005***	2·3 ± 0·1
Hoverflies (*Criorhina* sp.)	28·8 ± 2·4	18	**< 0·0005***	5·0
SVD vs. duration: $r = -0{\cdot}04$, $n = 389$, $P = 0{\cdot}47$. Split by visitor species: *B. terrestris* ($r = +0{\cdot}75$; $P < 0{\cdot}001$)				
Ipomoea (52·8)				
Bees	108·7	119	**< 0·0005***	76·0 ± 6·0
Andrena sp.	155·7 ± 15·9	19	**< 0·0005***	44·3 ± 9·8
Agapostemon sp.	118·5 ± 10·1	55	**< 0·0005***	103·6 ± 9·8
Partamona musarum	113·5 ± 9·4	11	**0·003***	50·9 ± 7·6
Tetragonisca angustula	70·4 ± 12·6	16	**0·008**	32·5 ± 3·4
Trigona fulviventris	35·8 ± 9·2	12	0·109	78·7 ± 18·2
Ants	65·0	37	**< 0·0005***	142·7 ± 13·7
Pseudomyrmex gracilis	69·0 ± 10·7	28	**0·001***	148·9 ± 15·5
Camponotus novograndensis	52·6 ± 12·1	9	0·068	123·3 ± 29·8
Beetles	93·5	40	**< 0·0005***	578·3 ± 86·9
Notoxus sp.	87·8 ± 9·8	36	**< 0·0005***	556·7 ± 92·1
SVD vs. duration: $r = -0{\cdot}14$, $n = 194$, $P = 0{\cdot}047$. Split by visitor species: *P. gracillis* ($r = -0{\cdot}48$; $P = 0{\cdot}010$)				
Heracleum (16·8)				
Hoverflies	43·7	239	**< 0·0005***	6·8 ± 0·3
Epistrophe grossulariae	61·8 ± 12·7	22	**< 0·0005***	7·1 ± 0·3
Episyrphus balteatus	55·8 ± 5·5	100	**0·005**	7·8 ± 0·4
Syrphus ribesii	32·1 ± 3·0	52	**< 0·0005***	2·7 ± 0·1
Eupeodes corollae	22·5 ± 4·0	12	**0·007**	10·0 ± 1·5
Platycheirus albimanus	25·8 ± 12·9	6	0·109	20
Other Syrphini sp.	28·0 ± 1·5	42	**< 0·0005***	10·2 ± 0·8
Other dipterans	80·5	152	**< 0·0005***	7·2 ± 0·5
Lucilia sericata	116·1 ± 12·8	33	**< 0·0005***	4·7 ± 0·1
Platypezidae sp.	79·9 ± 7·8	37	**< 0·0005***	3·5 ± 0·2
Anthomyiidae sp.	62·8 ± 19·8	6	0·068	1·7
Phaonia subventa	67·4 ± 8·1	76	**< 0·0005***	9·1 ± 0·7
SVD vs. duration: $r = -0{\cdot}04$, $n = 390$, $P = 0{\cdot}449$. Split by visitor species: *E. balteatus* ($r = +0{\cdot}23$; $P = 0{\cdot}032$), *L. sericata* ($r = -0{\cdot}40$; $P = 0{\cdot}020$), platypezid sp. ($r = +0{\cdot}54$, $P = 0{\cdot}001$)				
Rubus (52·7)				
Bees	256·2	42		44·3 ± 8·2
Bombus lucorum	343·3 ± 40·2	6	**0·026**	30·0 ± 3·4
Bombus terrestris	295·5 ± 53·2	16	**< 0·0005***	55·3 ± 10·3
Bombus pratorum	223·0 ± 82·5	7	0·068	77·1 ± 39·8
Bombus pascuorum	142·0 ± 21·2	5	**0·043**	12·2 ± 5·4
Apis mellifera	270·0 ± 49·8	4	0·068	12·5 ± 3·2
Hoverflies	136·6	35	**0·001***	99·9 ± 13·6
Rhingia campestris	172·6 ± 46·0	19	**0·005**	111·3 ± 14·3
Eristalis horticola	87·0 ± 40·6	5	0·317	14·8 ± 4·8
Episyrphus balteatus	80·0 ± 11·4	7	0·068	112·9 ± 26·0
Muscoid dipterans	54·6	13	0·180	67·8 ± 35·6
Wasps (*Vespula vulgaris*)	80·9 ± 8·2	6	0·066	21·5 ± 5·4
SVD vs. duration: $r = -0{\cdot}08$, $n = 92$, $P = 0{\cdot}428$. Split by visitor species: *B. terrestris* ($r = +0{\cdot}57$; $P = 0{\cdot}021$)				

STATISTICAL ANALYSES

Control pollen values for each plant species were subtracted from SVD values, with any resulting negative values set to zero for the purposes of statistical analyses. Since data for some plants were normally distributed but other data sets were not, nonparametric Wilcoxon Signed Ranks testing was used for consistency to compare SVD values with zero for each of the 13 plants. We show P levels as significant where they are below 0·05; Bonferroni corrections were routinely used, but since application of these is often regarded as too conservative, we merely indicate with an asterisk where they remain significant after Bonferroni corrections. SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, USA) was used for all statistical analyses.

Results

MEASURING SVD AND POLLINATOR EFFECTIVENESS

For every plant species studied, SVD values were calculated for 'visitor groups' defined according to traditional pollination syndromes (Willmer 2011), and for each visitor species separately where numbers of recordings allowed (Table 1; expanded details in Table S2). Those animal groups that a syndrome approach (Table S1) would predict as major pollinators generally had the highest SVDs, while for the more generalist plants several groups had high SVDs. For each one of the 13 species, the predicted syndrome was well matched with SVD findings, making SVD demonstrably a good measure of 'expected' pollinator effectiveness (PE). Of 105 plant–visitor combinations across the 13 plants, only 63 produced effective pollination.

TESTING PROXIES FOR POLLINATOR PERFORMANCE

Visit duration

Mean visit durations are included in Table 1, with Spearman's Rank Correlations (visit duration vs. SVD) for all visitors combined. Seven plant species showed no correlation, while the remaining 6 (*Malvaviscus, Helicteres, Cirsium, Centaurea, Knautia* and *Ipomoea*) showed a significant negative correlation. However, when visitor species were considered separately (Table 1) an overall relationship between SVD and visit duration was rarely preserved; duration could vary substantially *within* 'visitor groups', and *across* plant species for a given visitor, so was on its own an unreliable measure of PE.

Visit number or frequency

For *Agrimonia eupatorium,* visit numbers and rates, and hence pollinator performance for each major visitor, were calculated per hour and per day (Fig. 1). *Episyrphus balteatus* had the lowest SVD at the single-visit scale, but its high visitation rate gave it the highest SVD at per-hour and per-day scales; it would often be the 'best' pollinator. Conversely, *Rhingia campestris* had the highest SVD but the lowest per-hour and per-day SVD. But neither measure on its own gives a clear picture, whereas using visit frequency *with* SVD data can substantially affect the perception of 'most important pollinator' (cf. Olsen 1997; Ne'eman *et al.* 2010).

COMBINING VISIT DURATION, FEEDING TYPE AND VISITOR SPECIES WITH SVD MEASURES

A Generalized Linear Model was constructed (Table S3) to test the combined utility of typical measures of a good pollinator (visit duration, and type of feeding: nectar/pollen/both, or for *Byrsonima* oil/pollen/both) as proxies for pollination effectiveness; 'visitor species' was also included since variation in SVD between species but within functional groups is evidently important. In 8 of the 13 plants, the only factor significantly related to pollen deposition was visitor species, through its direct association with SVD; for the remaining species, other factors were inconsistently and rarely significant.

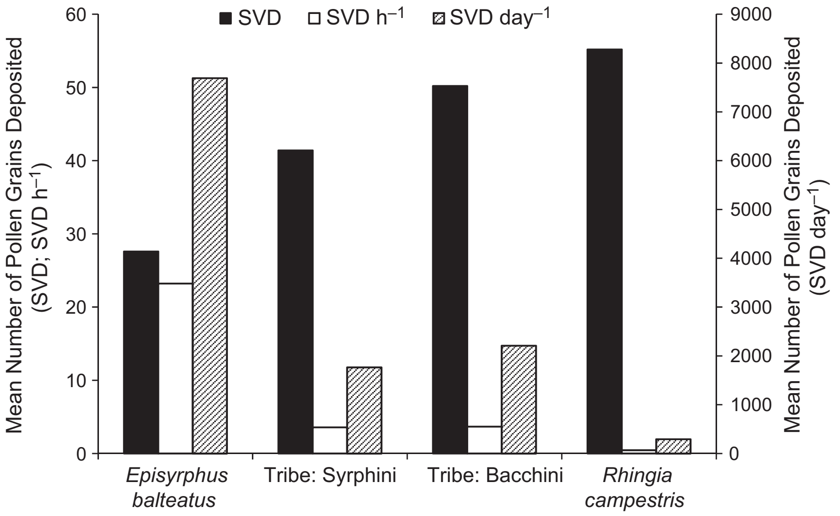

Fig. 1. Single-visit deposition (SVD) values for visitors to *Agrimonia eupatoria* scaled up to the 'per hour' and 'per day' level using visitation frequency data.

Discussion

Not all visitors are pollinators of a given plant species; a pollinator must deposit sufficient pollen on the correct and receptive stigma, and that pollen must be conspecific and viable. Our SVD protocols address the first two requirements, and any visibly heterospecific pollen grains were discounted. We show that SVD measures are relatively simple to incorporate into pollination studies, giving an accurate value for pollinator performance, and highlighting the effective visitors which in all 13 species largely correspond to expectations from a syndrome approach. Combined with visitation records, SVD can assess 'pollinator effectiveness' per hour, per day or per season, and can indicate 'pollinator importance', as with *Agrimonia*.

Only 63 of 105 plant–visitor interactions produced effective pollination (Table 2); and ineffective visits were not just the traditional 'illegitimate' visits, as many involved a normal route into the corolla by visitor species of similar size to the effective pollinators.

ARE PROXIES FOR SVD USEFUL OR APPROPRIATE?

Single-visit deposition is a good direct measure of PE; however, in most existing studies PE is not assessed, being substituted with other parameters such as visitor abundance, pollen load, number of stigma touches, feeding type or visit duration. Visitor abundance alone, though often used (e.g. Olsen 1997), is widely recognized as a poor measure of pollination outcomes (Johnson & Steiner 2000). A positive link may be recorded between abundance or visitation rate and pollen deposition, but can be weak [e.g. only 36% of variation in pollen deposition was explained thus for *Ipomopsis aggregata* (Engel & Irwin 2003)].

Abundance values for each animal and plant, and their interaction frequencies, can generate quantitative visitation networks, adding qualitative estimates of pollination using visitors' pollen loads (Popic, Wardle & Davila 2013); and assessing pollen fidelity (% conspecific pollen carried) can refine visitor importance further (Forup *et al.* 2008) and may encourage using visitor abundance and pollen load fidelity as proxies (Bosch *et al.* 2009; Kaiser-Bunbury *et al.* 2010). But pollen on visitors' bodies may poorly represent pollination potential; it can be deposited on incompatible or unreceptive stigmas, or lost before reaching another flower (Inouye *et al.* 1994; Harder & Routley 2006), so giving no correlation with pollen deposited on conspecific stigmas (Adler & Irwin 2006). Other possible proxies such as 'contact with reproductive structures' (Petanidou & Potts 2006; Gibson *et al.* 2011), number of stigma touches (Olsen 1997), measurements of visit duration (Fishbein & Venable 1996; Kaiser-Bunbury *et al.* 2010) and of pollen removal (Ivey, Martinez & Wyatt 2003) are similarly subject to problems of pollen loss. We therefore sought explicit relationships between these proposed measures and our direct SVD assessment.

CORRELATION OF VISIT DURATION AND POLLEN DEPOSITION

There were no significant correlations between visit duration and SVD for all visitors combined for 7 of our 13 species, but 6 showed a significant negative correlation (Table 1). In theory, longer visits could increase visitor contact with, and/or transfer of pollen to, a stigma; but they could also indicate 'ineffective' feeding (excessive grooming, eating pollen or floral tissues, avoiding anther or stigma contacts). SVD and PE will be higher for visitors which 'fit' the flower, feed rapidly on nectar and/or pollen, and quickly acquire body pollen. Short efficient visits will often predominate early on, when pollen is more abundant, and visitor groups show very different diurnal activity patterns (Willmer & Stone 2005). Thus, when visitor species are treated *separately* the correlations can change markedly, and only 3 of 13 species did not show such changes (Table 1). For the two ornithophilous plants (*Malvaviscus, Helicteres*), negative correlations disappeared, largely because visit

Table 2. Summary of visitor–pollinator analyses in relation to floral syndromes (ST, LT = short- or long-tongued). Further details on syndrome-related traits are in Supporting Material, Table 1

Plant	Syndrome based on traits	Functional groups of all visitors	Species of all visitors	Functional groups of pollinators	Species of pollinators	Species of ineffective visitors	Syndrome based on SVD analysis
Malvaviscus	Hummingbird	4	7	2	4	3	Hummingbird (*bee back-up*)
Helicteres	Hummingbird	2	4	2	4	0	Hummingbird (*bee back-up*)
Geranium	Bee	3	8	2	2	6	Bee
Digitialis	Bee	1	3	1	2	1	Bee
Byrsonima	Oil-bee	1	4	1	3	1	Oil-bee (*pollen-bee back-up*)
Agrimonia	Hoverfly	2	9	1	6	3	Hoverfly
Cirsium	LT bee/hoverfly	3	7	3	6	1	LT bee/hoverfly (*ST insect back-up*)
Centaurea	MT bee/hoverfly	1	4	1	3	1	LT bee/hoverfly (*ST insect back-up*)
Knautia	MT bee/hoverfly	3	9	3	8	1	LT bee/hoverfly (*ST insect back-up*)
Trifolium	LT bee/hoverfly	2	5	2	5	0	LT bee/hoverfly (*ST insect back-up*)
Ipomoea	Generalist/bee	6	15	3	6	9	Generalist/ST insect
Heracleum	Generalist	3	12	2	8	4	Generalist, smaller ST insect
Rubus	Generalist	4	18	3	6	12	Generalist, larger insects
All plant–visitor combinations		35	105	26	63	42	

duration and variance were low, and birds received the most pollen grains of any group. *Trifolium and Geranium* had significant overall negative correlations, but bumblebees showed significantly *greater* SVD in longer visits. In *Knautia,* with no overall relationship, *Rhingia campestris* showed a significant positive correlation and *Episyrphus balteatus* the opposite; these differing interactions are masked when visitor species are pooled.

Within all these comparisons, the common visitor species *E. balteatus* is instructive, showing positive or negative correlations between visit duration and SVD in different plants, though its mean visit duration did not vary greatly (Table 1). Evidently, the varying behaviour and PE of this species on each flower matters, rather than visit duration alone. This reinforces the problems with using visit duration as a proxy in its own right; no particular 'kind' of relation between visit duration and SVD can be assumed, for a visitor group or for a single visitor species.

COMBINED MEASURES AS PROXIES FOR POLLINATION EFFECTIVENESS

Our GLM showed that in 7 of 13 plant species the only factor significantly contributing to SVD was visitor species; feeding behaviour and visit duration were unimportant even where duration did affect pollen deposition (Table 1: *Malvaviscus*, *Helicteres*, *Ipomoea*). Duration and feeding behaviour never accounted for more than a small percentage of SVD variation, and in *Centaurea, Digitalis,* and *Geranium* no factor significantly explained SVD variation. Overall, in 11 of our 13 plants by far the largest predictor of variation in pollen deposition was visitor species.

POSSIBLE CRITICISMS AND DRAWBACKS OF SVD AND OF THIS STUDY

Firstly, measures of SVD are undoubtedly context-dependent, potentially affected temporally and spatially by environmental variation and relative species abundances. Hence, extrapolation between studies is dangerous, and SVD should be measured for a given interaction at a given site (as with many measures in pollination ecology, since phenology and rewards vary between sites).

Furthermore, SVD does not relate to the final female reproductive success of a flower, manifested in seed-set. But postpollination events have little to do with assessing pollinators, and reliance on seed-set may show the same effects described here (Spears 1983) or give contradictory results (Olsen 1997). Equally, SVD does not include estimates of pollen viability or germination, and some deposited pollen grains even though conspecific may not germinate, especially if large numbers clog up a small stigma.

Single-visit deposition measures may also be time-biased, tending to accentuate early visitors. Delayed removal of bags may help, so that 'first visits' occur later; but then an uncovered flower may have unusual rewards for that time of day, giving abnormal visit durations or frequencies. Elsewhere, we analyse time dependence of SVD more closely (King & Willmer, in prep.). We also note that all Scottish sites experienced very poor summer weather in 2008–2010 (high rainfall, poor sunshine), so visitor profiles were unusual: very low bee numbers (*Apis* and *Bombus*) occurred in eastern Scotland, and bees are under-represented in our data, with perhaps a concomitant increase in hoverfly numbers.

Finally, we considered just 13 plant species, and each in isolation, so proving that SVD methodology is feasible and timely for fieldwork, that it works with varying flower morphologies, and that measuring PE in this way is important because it shows up ineffective visitors. But the required and ongoing step is to use SVD to directly compare 'visitation' networks and true 'pollination' networks.

WHY DISTINGUISHING POLLINATORS AND VISITORS MATTERS

Flower visitors are not necessarily pollinators. Some are simple cheats, and their effects have been acknowledged (see Alarcón 2010; Genini *et al.* 2010). But eliminating obvious cheats is not enough: which apparently legitimate visitors correctly deposit significant pollen on stigmas? Some earlier studies (e.g. Wilson & Thomson 1991) made exactly this point but have been insufficiently built upon. More recent studies have paralleled our own in comparing visitor PE for just one plant genus (Kandori 2002; Stoepler *et al.* 2012), reaching similar conclusions regarding problems with proxies, and reinforcing the value of SVD (or a near equivalent) as a measure of effectiveness.

Without distinguishing visitors from pollinators, various negative consequences could ensue: conservation efforts could be misled by suggestions that networks are robust and extinctions can be tolerated (e.g. Memmott, Waser & Price 2004; Dupont *et al.* 2009; Hegland *et al.* 2010; Kaiser-Bunbury *et al.* 2010; Burkle & Alarcón 2011), or that visitors acting as 'hubs' or 'connectors' require most support (Olesen *et al.* 2007) whereas relationships between connectance and conservation value may be poor (Ruben, Devoto & Pocock 2012). Interpretations of specialization and generalization can also be seriously problematic when only visitation is recorded (see Alarcón 2010; Popic, Wardle & Davila 2013).

Single-visit deposition is a valuable simple and direct means of measuring pollinator effectiveness, for which indirect proxies are unreliable. Here, variation in SVD was poorly related to visit duration or feeding behaviour, but strongly explained by visitor species, the most effective visitors being those predicted as the most important pollinators from syndrome-related floral traits. We are now incorporating SVD into networks to extend this argument; we urge care over extrapolations from existing 'pollinator' networks, particularly where these are used to infer consequences for ecosystem management and for modelled extinction threats.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Observed floral traits for each plant species, and available literature, to support our ascription of pollination syndrome for each of our 13 plants.

Table S2. Mean SVD values (±SE) for visitor groups, and all visitor species, for 13 plant species, with significance indicated as the difference in SVD (corrected for mean pollen on unvisited control flowers, value in parentheses below plant name) from zero.

Table S3. Summary of results of General Linear Models for each of the 13 plant species, considering the relationship between visitor species, duration of visit and feeding type and the single-visit stigmatic pollen deposition (SVD).