PLANT-POLLINATOR INTERACTIONS FROM FLOWER TO LANDSCAPE

The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks

Steven D. Johnson[*,1], **Marcela Moré**[2], **Felipe W. Amorim**[3], **William A. Haber**[4], **Gordon W. Frankie**[5], **Dara A. Stanley**[1], **Andrea A. Cocucci**[2] **and Robert A. Raguso**[6]

[1]*School of Life Sciences, University of KwaZulu-Natal, P Bag X01, Scottsville, Pietermaritzburg 3209, South Africa;* [2]*Laboratorio de Ecología Evolutiva y Biología Floral, Instituto Multidisciplinario de Biologıa Vegetal (CONICET-UNCba), CC 495, CP 5000, Cordoba, Argentina;* [3]*Instituto de Biociências, Universidade Estadual Paulista 'Júlio de Mesquita Filho', Caixa Postal 510 CEP 18618-689, Botucatu, São Paulo, Brazil;* [4]*Apartado Postal 50-5655, Monteverde, Costa Rica;* [5]*Department of Environmental Science, Policy, & Management, UC Berkeley, 130 Mulford Hall #3114, Berkeley, CA 94720, USA; and* [6]*Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA*

Summary

1. Proboscis length has been proposed as a key dimension of plant pollination niches, but this niche space has not previously been explored at regional and global scales for any pollination system. Hawkmoths are ideal organisms for exploring pollinator niches as they are important pollinators in most of the biodiverse regions of the earth and vary greatly in proboscis length, with some species having the longest proboscides of all insects.

2. Using data sets for nine biogeographical regions spanning the Old and New World, we ask whether it is possible to identify distinct hawkmoth pollination niches based on the frequency distribution of proboscis length, and whether these niches are reflected in the depths of flowers that are pollinated by hawkmoths. We also investigate the levels of specialization in hawkmoth pollination systems at the regional and community level using data from interaction network studies.

3. We found that most regional hawkmoth assemblages have bimodal or multimodal distributions of proboscis length and that these are matched by similar distributions of floral tube lengths. Hawkmoths, particularly those with longer proboscides, are polyphagous and at the network level show foraging specialization equivalent to or less than that of bees and hummingbirds. In the case of plants, shorter-tubed flowers are usually visited by numerous hawkmoth species, while those that are longer-tubed tend to exclude shorter-proboscid hawkmoths and thus become ecologically specialized on longer-proboscid hawkmoth species. Longer-tubed flowers tend to have greater nectar rewards, and this promotes short-term constancy by long-proboscid hawkmoths.

4. Our results show that pollinator proboscis length is a key niche axis for plants and can account for the patterns of evolution in functional traits such as floral tube length and nectar volume. We also highlight a paradoxical trend for nectar resource niche breadth to increase according to proboscis length of pollinators, while pollinator niche breadth decreases according to the tube length of flowers.

Key-words: adaptive radiation, biogeography, co-evolution, community ecology, ecological shifts, floral adaptation, long-tongued, nectar, Sphingidae

Introduction

Ecological niches have two important consequences for biodiversity. In the ecological sense, niches determine where species occur and whether they coexist (Chase & Leibold 2003; Silvertown 2004). In the evolutionary sense, niches shape traits that are required for niche occupancy (Losos 2010). Indeed, trait modifications often provide valuable clues about the existence of niches in a habitat. For example, diversification in bill morphology of birds (Galapagos finches being the most famous example) can

*Correspondence author. E-mail: Johnsonsd@ukzn.ac.za

provide clues about the existence of different feeding niches in the habitats occupied by these birds (Grant & Grant 1982). Similarly, particular combinations of floral traits can provide an indication of the availability of pollinator niches (Fenster *et al.* 2004). Pollinators are niches for plants in the sense that they represent ecological opportunities that can be exploited for the enhancement of fecundity (Johnson 2010). Sampling of assemblages of animal flower visitors can also provide direct information about the availability of pollinator niches in communities (Ackerman 1985; Haber & Frankie 1989). Because specialization for occupancy of particular niches shapes trait evolution (Devictor *et al.* 2010), ecological shifts between niches are believed to be a major driver of adaptive radiation in plants and animals (Schluter 2000; Losos & Mahler 2010). Here, we focus specifically on the role of hawkmoth pollinator niches in modifications of plant floral traits at local and global scales.

Hawkmoths are ideal subjects for examining the importance of proboscis length as a niche axis for plants because proboscis length varies widely (from 1 to 28 cm) in this insect group (Miller 1997; Kitching & Cadiou 2000) and because a large number of unrelated plant species across the globe show convergent evolution of flowers that are specifically adapted to these insects (Silberbauer-Gottsberger & Gottsberger 1975; Grant 1983; Martins & Johnson 2013; Amorim, Wyatt & Sazima 2014) (Fig. 1). Selection on floral tube length of several plant species has been shown to be mediated by morphometric interactions with the proboscis length of hawkmoths (Nilsson 1988; Alexandersson & Johnson 2002; Moré *et al.* 2012).

The moth family Sphingidae comprises about 1400 species and 200 genera world-wide (Kitching & Cadiou 2000; Kawahara *et al.* 2009). About 70% of these species occur in Africa (Old World) or the Americas (New World) (Schreiber 1978). Biogeographical regions mostly have distinct hawkmoth faunas. For example, of the *c.* 56 genera that occur in the Neotropics, only six are shared with the Old World and about 30 with the Nearctic region (Kitching 2016). Adults of most hawkmoth species bear functional proboscides and feed from flower nectar (Fig. 2a–f). However, some species lack proboscides or have exceedingly short ones and may not feed as adults. Except for some strictly diurnal hawkmoths, such as species of *Aellopos*, *Hemaris* and *Macroglossum*, most species are crepuscular and nocturnal.

Phylogenies based both on morphological and on molecular data have confirmed the existence of three monophyletic clades – Smerinthinae, Sphinginae and Macroglossinae – within the Sphingidae (Kawahara *et al.* 2009). Species with longer proboscides are restricted to the Sphinginae, and the first lineage to branch off in this clade contains the palaeotropical genus *Xanthopan* and a Neotropical group consisting of *Amphimoea*, *Cocytius* and *Neococytius* (Kitching 2002; Kawahara *et al.* 2009). This lineage includes three species with proboscides longer than 20 cm. Old and New
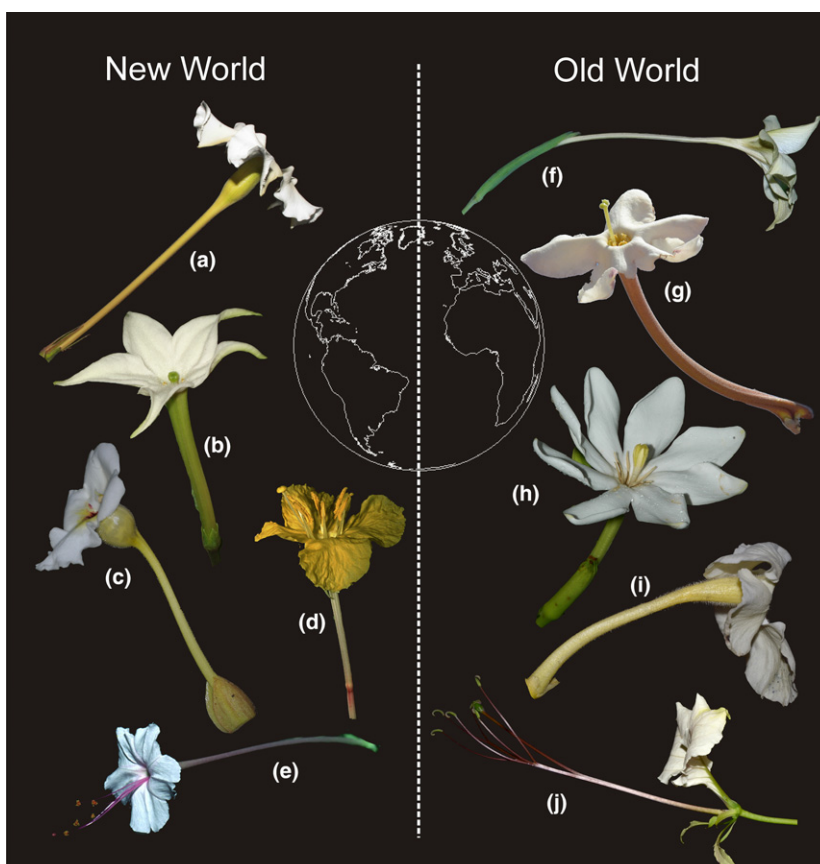

Fig. 1. Representative examples of hawkmoth-adapted flowers showing convergent evolution across continental land masses. (a) *Mandevilla longiflora* (Apocynaceae). (b) *Jaborosa integrifolia* (Solanaceae). (c) *Craniolaria argentina* (Martyniaceae). (d) *Calylophus tourneyi* (Onagraceae). (e) *Mirabilis longiflora* (Nyctaginaceae). (f) *Gladiolus longicollis* (Iridaceae). (g) *Sesamothamnus lugardii* (Pedaliaceae). (h) *Gardenia thunbergii* (Rubiaceae). (i) *Harveya speciosa* (Orobanchaceae). (j) *Cladostemon kirkii* (Capparaceae).
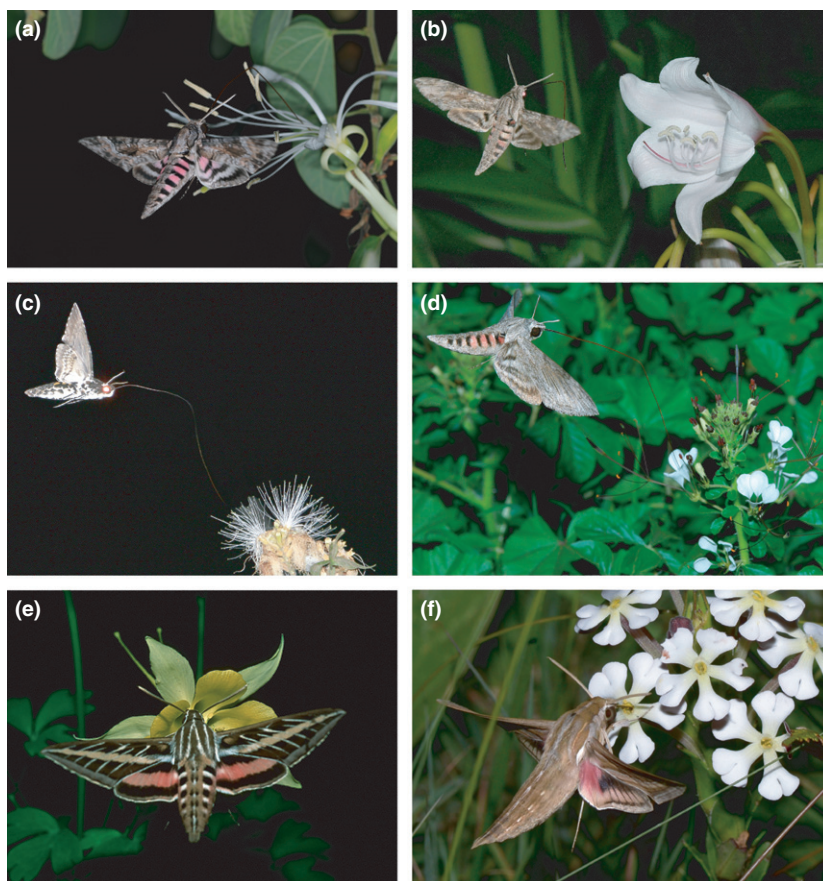
Fig. 2. Examples of interactions between hawkmoths and flowers. (a and b) Long-proboscid hawkmoths effectively pollinate flowers with a depth that matches the proboscis length. (a) *Agrius cingulata* on *Bauhinia mollis* (Fabaceae) in Argentina. (b) *Agrius convolvuli* on *Crinum macowanii* (Amaryllidaceae) in South Africa. (c and d). Long-proboscid hawkmoths are generalist foragers that regularly obtain nectar from short-tubed flowers. (c) *Manduca rustica* on *Inga vera* (Fabaceae) in Brazil. (d) *Agrius convolvuli* on *Gynandropsis gynandra* (Brassicaceae) in Kenya. (e and f) Short-tongued hawkmoths are only able to obtain nectar from short-tubed flowers. (e) *Hyles lineata* on *Aquilegia chrysantha* (Ranunculaceae). (f) *Hippotion celerio* on *Zaluzianskya natalensis* (Scrophulariaceae) in South Africa.

World hawkmoths in this lineage may share long proboscides through common descent rather than independent evolution. Since hawkmoths likely have an Old World origin (Kawahara *et al.* 2009), it is plausible that some of the hawkmoths in this group that colonized the New World already possessed long proboscides, particularly so since long-proboscid hawkmoths are polyphagous and do not strictly depend on long-tubed flowers for nectar (Haber & Frankie 1989; Martins & Johnson 2013; Amorim, Wyatt & Sazima 2014).

The first pieces of the puzzle of whether there is matching of hawkmoth proboscis and flower tube lengths were obtained independently from the Old and New World. Darwin (1862) famously predicted the existence of a long-proboscid hawkmoth in Madagascar based on the extremely long (*c.* 29 cm) floral spur of the star orchid *Angraecum sesquipedale* (Arditti *et al.* 2012). Soon afterwards, Hermann Müller reported the existence of a hawkmoth with a 25-cm proboscis discovered by his brother Fritz in the south of Brazil (Müller 1873). This moth was most likely a specimen of *Amphimoea walkeri* or *Neococytius cluentius* (Moré, Kitching & Cocucci 2005). We now know that the length of the spur of Darwin's orchid matches that of the Malagasy hawkmoth *Xanthopan morganii* subsp. *praedicta* and that there are flowers of plant species in South America that match the proboscis of Müller's hawkmoth (Silberbauer-Gottsberger & Gottsberger 1975). These species represent the extremes of hawkmoth and flower dimensions. What are much less well known is the actual distributions of hawkmoth proboscis and floral tube lengths across the world, and the consequences of the dimensions of these traits for the levels of specialization in pollination systems.

While proboscis length is a plausible niche axis for plants, it should be borne in mind that like many other niches in nature, proboscis length niches arose gradually by historical biotic interactions. Despite some suggestions to the contrary (Wasserthal 1997), co-evolution with plants was almost certainly responsible for the evolution of very long proboscides in hawkmoths (Nilsson 1998), as has been shown empirically for other insect–plant associations (Anderson & Johnson 2008; Toju 2008; Pauw, Stofberg & Waterman 2009). However, it is also very likely that the vast majority of extant hawkmoth-pollinated plants have adapted to existing proboscis lengths of hawkmoths by a process of ecological shifts from other pollinators, including hawkmoths with shorter proboscises (Wasserthal 1997; Whittall & Hodges 2007).

Recent studies in Africa suggest that long-proboscid hawkmoths comprise a niche for plant pollination that is distinct from the niche for pollination by short-proboscid hawkmoths. This is evident from the bimodal distribution of hawkmoth proboscis lengths with modes at *c.* 4 cm and *c.* 10 cm, and a corresponding bimodal distribution in the

flower tube lengths of hawkmoth-adapted flowers (see Martins & Johnson 2013; Johnson & Raguso 2016). Studies in the New World and Madagascar have suggested a more complex picture with more even distributions of proboscis length across species (Nilsson *et al.* 1985; Agosta & Janzen 2005; Sazatornil *et al.* 2016). Given the importance of hawkmoth pollination in tropical and subtropical regions of Africa and the Americas, we considered it opportune to review the morphometrics of hawkmoth pollination systems at a global scale. In addition, there is a growing amount of data on interactions between hawkmoths and plants, allowing the assessment of the relationships between trait values and levels of specialization in these interactions. Existing studies suggest that hawkmoths are highly polyphagous and readily feed on flowers which have tubes much shorter than their proboscis lengths (Fig. 2c,d), while plants adapted to hawkmoths are more specialized, particularly when long-tubed (Nilsson *et al.* 1987; Haber & Frankie 1989; Martins & Johnson 2013; Amorim, Wyatt & Sazima 2014). To determine whether hawkmoth pollination systems are generally characterized by asymmetry between generalist pollinators and specialist plants, we collated existing studies of interactions between hawkmoths and plants. These included studies of interaction networks involving hawkmoths and plants at the level of local communities. Network studies allow the calculation of measures of interaction specialization that take into account estimates of the availability of species that can potentially interact. Different insights are obtained from absolute measures of specialization, such as the number of species with which a given species interacts vs. measures that are standardized and take local assemblages into account (Kay & Schemske 2004; Maglianesi *et al.* 2014). We use both approaches in this study and feel that the merits of each approach depend on the particular questions posed and the spatial scale of the analyses. For example, niche breadth is best studied using absolute measures of specialization at the regional scale, while niche specialization is best studied using frequencies of interactions at the local community level.

The overall aim of this study was to assess the structure of hawkmoth pollination niches and hawkmoth–plant interaction networks world-wide. To do so, we addressed the following specific questions: (i) 'Are regional hawkmoth assemblages characterized by unimodal or multimodal frequency distributions of proboscis length?' (ii) 'Do the tube lengths of night-opening flowers correspond to the frequency distribution of hawkmoth proboscis lengths at the regional and global scales?' (iii) 'Are longer-tubed night-opening flowers visited by fewer hawkmoth species with longer proboscides, and do they offer greater nectar rewards than shorter-tubed night-opening flowers?' (iv) 'What is the structure of hawkmoth–plant visitation networks (in terms of overall specialization and hawkmoth foraging specialization), and how do levels of specialization in these networks vary across biogeographical regions and compare with networks involving other functional groups of flower visiting animals, such as bees and hummingbirds, as well as entire assemblages of flower visitors?'

Materials and methods

STUDY SITES

The analyses in this paper are based on data sets on hawkmoth–plant interactions at 11 sites representing nine biogeographical regions distributed across the Old and New Worlds. The Old World sites were Mpala and Kitengela representing the drylands region in Kenya (Martins & Johnson 2013), subtropical South Africa (Johnson & Raguso 2016) and Madagascar (Nilsson *et al.* 1985, 1987; Baum 1995). The New World sites were the Sonoran Desert in North America (Alarcón, Davidowitz & Bronstein 2008), Guanacaste dry forest in Costa Rica (Haber & Frankie 1989), Cerrado (Neotropical savanna) and Atlantic Rainforest in the south-east of Brazil (Amorim *et al.* 2009; Amorim, Wyatt & Sazima 2014) and Chaco montane dry woodland and two transitional zones (CY1 and CY2) between western Chaco woodland and Yungas montane rain forest in Argentina (Moré 2008; Sazatornil *et al.* 2016). Existing data sets for these regions were supplemented with additional previously unpublished data collected by the authors. Data sets not published elsewhere are available in the Supporting Information.

The analyses in this study were conducted at different spatial scales depending on the questions that are posed and the availability of data. Analysis of morphometric niches was based on the level of biomes or vegetation types. Correlates between floral tube length and hawkmoth diversity and nectar properties were conducted at the regional biogeographical scale. Analyses of interaction networks were conducted at the level of local communities since the metrics for these analyses assume that all species can potentially interact.

REGIONAL HAWKMOTH FAUNAS AND PLANT FLORAS

To establish the frequency distribution of hawkmoth proboscis length in each biome or vegetation type, we used data from light-trapping surveys, apart from the Kenya drylands where we used a survey of hawkmoths netted on flowers. Sampling details are given in the source references provided above. In total, we obtained data for 7228 individual hawkmoths. The number of hawkmoth individuals recorded per region varied from 386 to 1380 (median = 807), and the number of plant species ranged from 9 to 63 (median = 27). We measured hawkmoth proboscis length from all captured moths to the nearest 0·1 mm with a digital calliper or to the nearest 1 mm with a steel ruler, or used the values provided by authors that used the same measurement protocols. Nomenclature and classification of hawkmoths follow Kitching (2016). The frequency distribution of hawkmoth proboscis lengths was plotted at 1-cm intervals for species and individuals in each region.

For each region, we compiled assemblages of plant species that are likely to be hawkmoth-pollinated. This was done using previously published studies, direct observations and pollen load analyses as described below in the section on network analyses, and floral syndrome traits for species in regional floras in different herbaria (CORD, LIL and SI in Argentina, HUFU and UEC in Brazil) and systematic monographs. For the latter, we considered flowers that are pale, scented, night-opening and have a long (>2 cm) narrow floral tube likely to be adapted for hawkmoth pollination. Flower length was measured to the nearest 1 mm using a digital calliper or steel ruler for at least three flowers per plant and ten plants per species when their abundance allowed it. We measured flower length as corolla tube length in tubular and salverform flowers or as the distance between stigma and nectar in brush-type

and funnel-shape flowers. Regional assemblages of putative hawk-moth-pollinated plant species varied from 9 to 252 species (median = 48). For each region, we plotted the frequency distribution of floral tube lengths for these species at 1-cm intervals.

To assess whether the frequencies of hawkmoth proboscis lengths and flower tube lengths were significantly multimodal (an indication of different niches), we used Hartigan's diptest (Hartigan & Hartigan 1985), which is a nonparametric test for multimodality of the data. As the diptest is known to produce very conservative *P* values (Xu *et al.* 2014), we also used finite mixture models which determine the number of Gaussian distributions that best account for the structure of the data. Finite mixture models do not strictly test for multimodality, but multimodal distributions tend to be best fitted by models with more than one Gaussian distribution (Gowell, Quinn & Taylor 2012). Model selection was based on AICc values and models that differed by more than 10 units from the best-fitting model (lowest AICc value) were considered to lack support (Burnham & Anderson 2004). Hartigan's diptest was implemented in the DIPTEST package (Maechler & Ringach 2015) in R (version 3.3.1; R Core Team, 2014), and the finite mixture models were implemented in PAST (Hammer, Harper & Ryan 2001).

RELATIONSHIPS BETWEEN TRAITS AND ECOLOGICAL SPECIALIZATION

To investigate the relations between floral tube length and the size and composition of hawkmoth visitor assemblages at regional and global scales, we used data obtained from the community network studies described below, supplemented with previously published studies of hawkmoth pollination of plants. For this purpose, we updated a survey by Grant (1983) to include more recently published studies (Table S1, Supporting Information). We used generalized linear models implemented in SPSS 23 (IBM Corp., Armonk, NY, USA) to explore the relations between floral tube length and hawkmoth visitor assemblages. Models predicting the number of hawkmoth visitor species as a response variable incorporated a Poisson distribution and log-link function, while models predicting the proportion of hawkmoth visitor species with long proboscides (>6 cm) incorporated a binomial distribution and logit-link function. These models included floral tube length as a covariate and the fixed factors, World (Old vs. New), site (nested within World) and the interaction between World and tube length. Significance in all generalized linear models was assessed using likelihood ratio tests.

We investigated the relationships between the proboscis length of hawkmoth species and foraging specialization (in terms of the number of plant species that they are known to visit) using linear regression analysis based on data from the network studies described below and published studies, where applicable. Analysis of the general relationships between proboscis length of hawkmoth species and foraging specialization was conducted using analysis of covariance (ANCOVA). This analysis did not include World (Old vs. New) because community-level data for hawkmoth specificity in the Old World were available for Kenya only. The ANCOVA included community as a fixed factor, proboscis length as a covariate and the interaction between community and proboscis length.

COVARIATION OF FLORAL TUBE LENGTH AND NECTAR REWARDS

We obtained data for floral nectar rewards of 110 plant species visited by hawkmoths. These data were obtained from previously published studies or from our own previously unpublished measurements (Fig. S2). The volume of nectar in unvisited newly open flowers (typically 3–10 from each of 10 plants per species) was measured using graduated microlitre syringe (Hamilton, NV,

USA) or micropipettes and the sugar concentration established using 0–50% refractometers (e.g. Atago, Tokyo, Japan). The amount of sugar available per flower was calculated using the methods mentioned in Cruden & Hermann (1983). Univariate relationships between nectar sugar content and floral tube length were explored using linear regression analysis. Global models testing nectar volume and nectar sugar content as a response variable incorporated a Gaussian distribution and identity link function. These models included floral tube length as a covariate and the fixed factors, World (Old vs. New), site (nested within World) and the interaction between World and tube length.

NETWORK ANALYSES

For nine of the 11 study sites, interactions between hawkmoths and flowers were recorded at the level of a local community, allowing us to construct quantitative hawkmoth–plant interaction networks (Table 1). Interactions were established by pollen load analysis and by direct observations and netting or photography of hawkmoths visiting flowers. For pollen load analyses of hawkmoths sampled in light traps, individuals were inspected under a stereomicroscope to locate pollen loads that were then mounted in glycerine jelly or treated by acetolysis for later study (Kearns & Inouye 1993). Pollen identifications were made by comparison with reference samples taken from flowering plants present in each community (Kislev, Kraviz & Lorch 1972) or with pollen identification guides (Markgraf & D'Antoni 1978). Interaction data were converted into matrices representing the number of recorded interactions between each hawkmoth and plant species. Across these networks, the number of hawkmoth species ranged from 3 to 55 (median = 14) and the number of plant species ranged from 9 to 34 (median = 19). Six networks were based on pollen load analysis of light-trapped hawkmoths to record hawkmoth–plant interactions indirectly, while two networks were based on the direct observations of moths visiting flowers, and one network (from Costa Rica) was assembled using both approaches (Table 1). For the latter network (Table S2), the visitation data represented a smaller network (113 interactions) than the one based on pollen analysis (526 interactions). However, both pollen and visitation networks captured a number of unique interactions (Table S3) and were therefore combined for analysis.

We calculated network specialization using the index H_2' (Blüthgen, Menzel & Blüthgen 2006). This index ranges from 0 (most generalized) to 1 (most specialized). To assess whether the values of H_2' obtained for each network differed significantly from random networks, we used the null model method described by Blüthgen, Menzel & Blüthgen (2006). This was based on 10 000 randomizations and was carried out using software available online (http://rxc.sys-bio.net/).

We measured the specialization of flower visitors in two main ways. First, we calculated unweighted generality as the mean number of plant species with which a hawkmoth species interacts. Secondly, we calculated hawkmoth specialization (d_i') for each hawkmoth species using the standardized Kullback–Leibler distance, which compares the distribution of the interactions with each partner (in this case plants) to partner availability (Blüthgen, Menzel & Blüthgen 2006). We also calculated an overall measure of specialization for the entire hawkmoth community in each network ($<d'>$), using a mean value of the standardized index d_i' weighted by the number of hawkmoth interactions as an estimate of abundance, since independent measures of abundance were not available (Blüthgen, Menzel & Blüthgen 2006). All network analyses were carried out in the BIPARTITE package (v. 2.05; Dormann *et al.* 2009) in R (version 3.1.0; R Core Team, 2014) using the network-level (Dormann, Gruber & Fruend 2008) and species-level (Dormann 2011) commands.

To determine whether the levels of specialization vary among networks involving different pollinator groups, we compared the

Table 1. Location, summary data and references for the nine hawkmoth–flower visitation networks included in this study. All networks were quantitative: s = sampling method, hm = the number of hawkmoths, pla = the number of plant species, H_2' = a measure of ecological specialization in networks, $<d'>$ = a measure of hawkmoth specialization, gen = unweighted generality. For all networks, apart from the Sonoran Desert network that was too small for calculations, values of H_2' differed significantly ($P < 0.0001$) from a random network

Realm	Habitat	Location	s*	hm	pla	H_2'	$<d'>$	gen	Reference
Old World	Acacia bush	Mpala, Kenya	o	10	15	0·54	0·45	3·3	Martins & Johnson (2013)
	Grassland	Kitengela, Kenya	o	12	19	0·57	0·56	3·5	Martins & Johnson (2013)
New World	Semi-arid grassland	Sonoran Desert, USA	p	3	9	0·20	NA	7	Alarcón, Davidowitz & Bronstein (2008)
	Atlantic Forest	Brazil	p	39	34	0·24	0·27	4·26	Sazatornil et al. (2016)
	Cerrado	Brazil	p	40	27	0·22	0·22	4·8	Sazatornil et al. (2016)
	Transition zone CY1[†]	Argentina	p	14	17	0·18	0·16	5·21	Sazatornil et al. (2016)
	Transition zone CY2[†]	Argentina	p	16	14	0·17	0·15	4·63	Sazatornil et al. (2016)
	Chaco Montane woodland	Argentina	p	8	22	0·16	0·16	10·7	Sazatornil et al. (2016)
	Tropical dry forest	Guanacaste Costa Rica	p and o	55	27	0·30	0·25	4·92	Table S2

*Type of sampling: p = pollen networks based on the identification of pollen grains on bodies of hawkmoths captured using light trapping, o = direct observations of hawkmoths observed visiting flowers.
[†]Transition zone refers to the transition between Chaco woodland and Yungas forest.

values of H_2' for the nine hawkmoth–plant networks with those for hummingbird–plant ($n = 16$), bee–plant ($n = 15$) and complete visitor–plant interaction networks in which all flower visitors were recorded ($n = 35$). We obtained metrics for the hummingbird and complete networks presented in Schleuning et al. (2012). Bee–plant networks were obtained by extracting bee–plant interaction data from 15 quantitative networks from the Interaction Web Database (https://www.nceas.ucsb.edu/interactionweb/index.html; accessed 21 March 2016) and other sources (see Table S4; some networks from the data base had low numbers of bees and so these were not included). To compare hawkmoth specialization to that of other pollinator groups, we used measures of unweighted generality and $<d_i'>$ for each network. We tested for the differences in specialization among networks and pollinator groups using analysis of variance with Dunn–Šidák post hoc tests for comparisons of means.

Results

REGIONAL HAWKMOTH FAUNAS AND PLANT FLORAS

We found a remarkable degree of matching between the frequency distributions of proboscis length in hawkmoths and floral tube length in plants across the various regions included in this study (Fig. 3). Trait distributions appear bimodal for moth and plant assemblages in the Sonoran Desert of North America, the Brazilian Cerrado and in Kenya and South Africa on mainland Africa. Trait distributions appear multimodal and less sharply defined for the remainder of the regions in Central and South America and in Madagascar. Using Hartigan's diptest, we found very strong statistical support for deviations from unimodality for all assemblages of hawkmoth individuals and for several plant assemblages (Table S5). Finite mixture models with a single Gaussian component were not supported for any assemblages, while models with two to four Gaussian components were generally well supported (Table S5). By visual inspection, the frequency distributions of proboscis lengths of hawkmoth individuals (thus taking the abundance of different species into account) matched those of floral tube lengths better than did the frequency distributions of the mean proboscis lengths of hawkmoth species (Fig. 3).

RELATIONSHIPS BETWEEN TRAITS AND ECOLOGICAL SPECIALIZATION

The number of hawkmoth visitor species, an absolute measure of ecological specialization, declined significantly with increasing floral tube length in five of the seven regions included in this study (Fig. 4). The number of hawkmoth species recorded per plant varied significantly among regions and was negatively associated ($\beta = -0.092$) with floral tube length (Table S6). The proportion of hawkmoth visitors with long proboscides (>6 cm) increased significantly with floral tube length in five of the seven regions for which these data were available (Fig. 5). The proportion of visitors with long proboscides increased positively ($\beta = 0.485$) with floral tube length and was significantly higher in the Old World (42%) than in the New World (35%) and also differed significantly among regions (Table S6). The significant interactions between World and tube length (Table 1) reflect the steeper rise in the fraction of long-proboscid moths with tube length in the Old World compared with the New World.

The number of plant species known to be visited by hawkmoths showed a significant positive relationship with proboscis length at some sites (Fig. S1). In an ANCOVA model involving the six sites for which these data were available, there was no significant difference ($F = 1.84$, $P = 0.11$) among sites in the number of plant species visited by moths (means ranged from 4·0 to 5·1 plant species per hawkmoth species). However, there was a significant positive association ($\beta = 0.121$) overall between proboscis length and plant species visited ($F = 11.4$, $P = 0.01$), and a significant interaction between site and proboscis length ($F = 2.81$, $P = 0.018$), implying geographical variation in
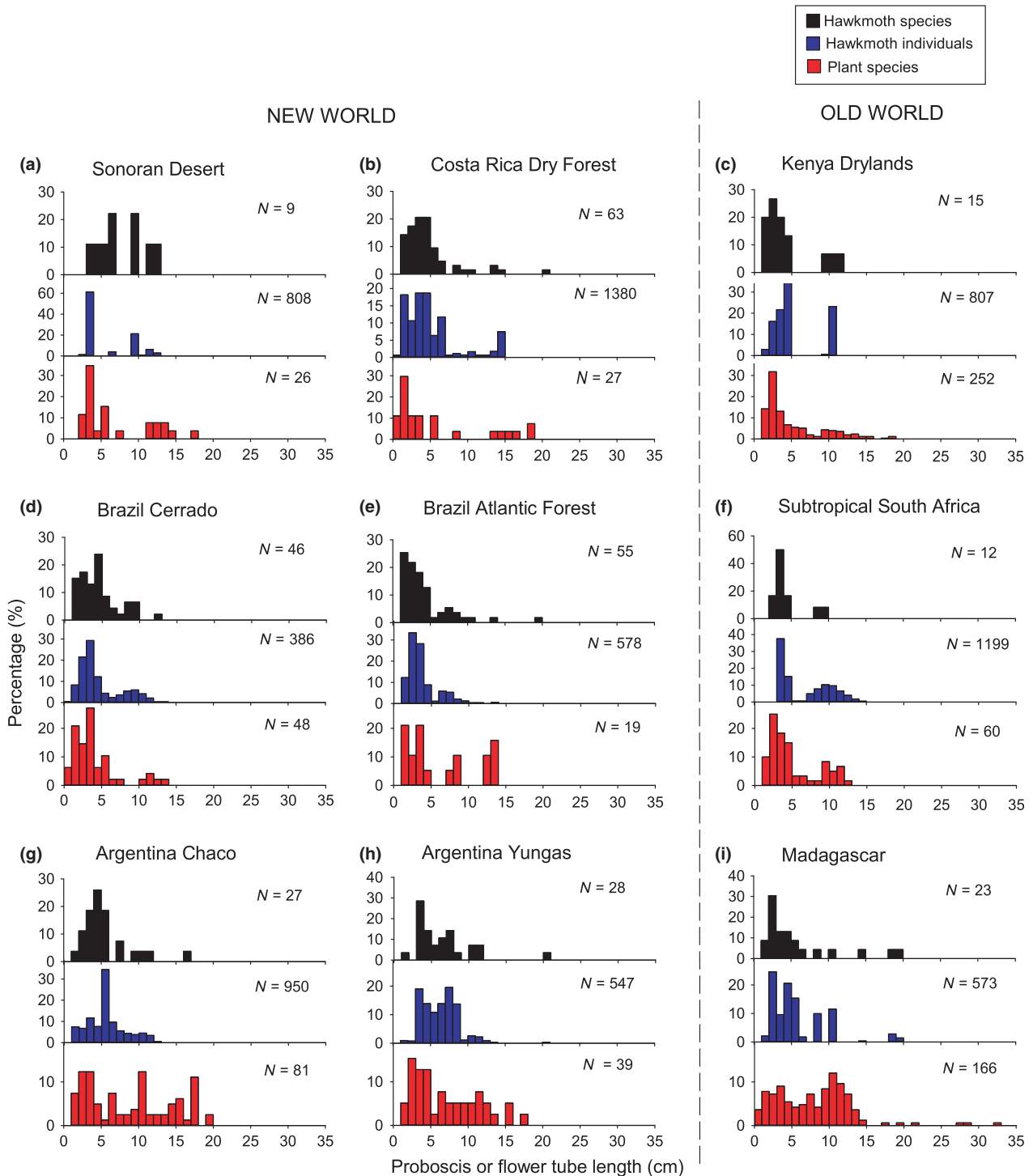
Fig. 3. Frequency distributions for proboscis length of hawkmoth species (black bars, upper panel), hawkmoth individuals (blue bars, middle panel) and plant species (red bars, lower panel) in various biogeographical regions of the Americas and Africa (including Madagascar).

the slopes of the relationships between hawkmoth proboscis length and plant species visited.

COVARIATION OF FLORAL TUBE LENGTH AND NECTAR REWARDS

Nectar volume was positively correlated with floral tube length for six of the seven regions (Fig. 6). A highly

significant positive relationship ($\beta = 2\cdot433$) between floral tube length and nectar sugar content was also evident in the global model (Table S6). Very similar relationships were evident for nectar sugar content, which was positively correlated with floral tube length for six of the seven regions (Fig. S6), and showed a general positive relationship with tube length (Table S6). The only region in which these relationships were not significant was Madagascar, which had
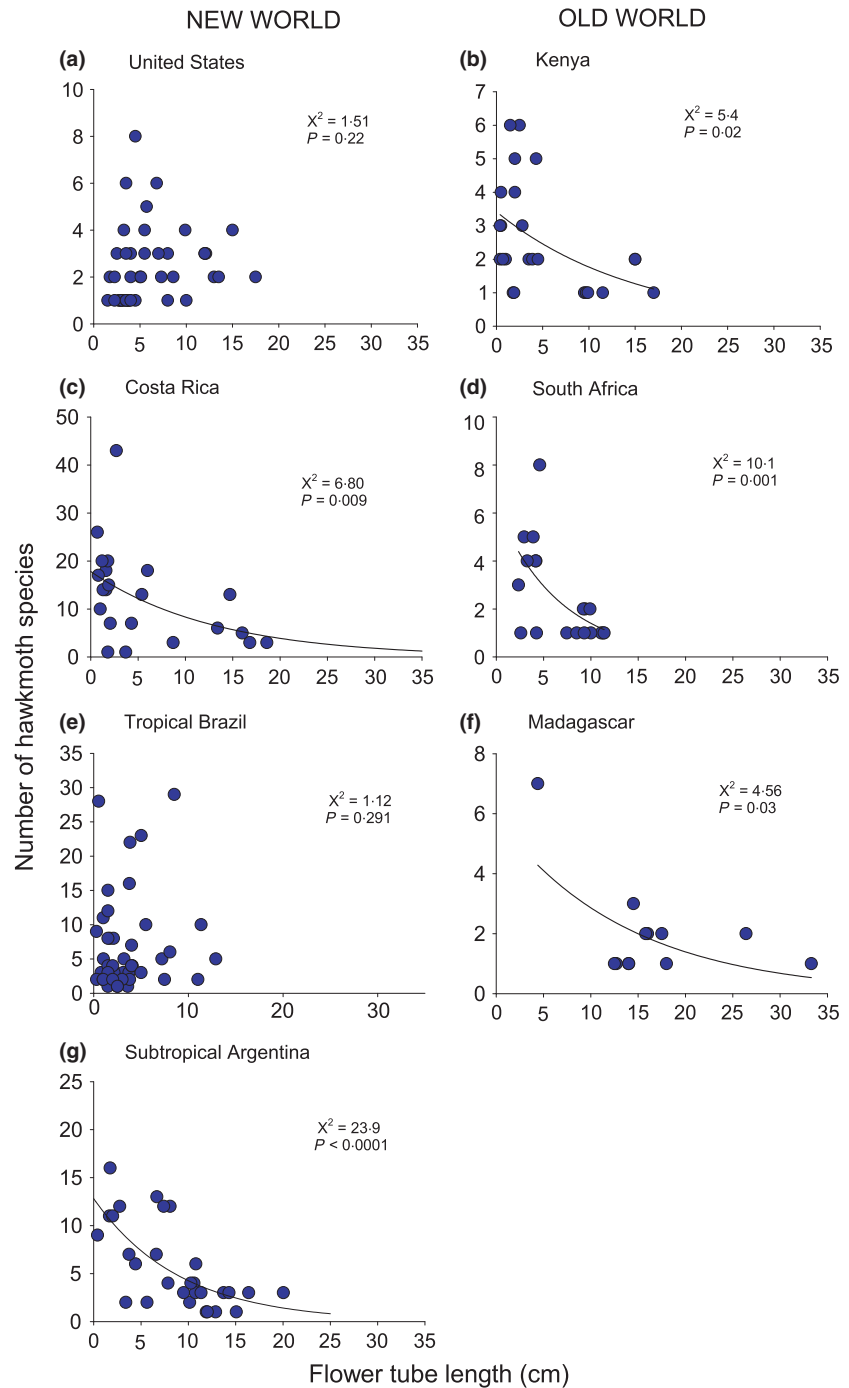
NEW WORLD   OLD WORLD

Fig. 4. The relationships between floral tube length and the diversity of hawkmoth visitor species in various regions of the Old and New World.

the fewest available nectar data for plants visited by hawkmoths.

NETWORK ANALYSES

Overall levels of specialization (H_2') varied significantly among networks involving different pollinator groups ($F_{3,69} = 10.1$, $P < 0.001$). The mean value of H_2' for hawkmoth–plant networks (0.287 ± 0.047) was significantly lower than that for bee–plant networks (0.59 ± 0.036) and

whole networks (0.48 ± 0.024), but did not differ from that for hummingbird–plant networks (0.42 ± 0.036).

There was a significant variation in unweighted generality between pollinator groups ($F_{3,69} = 10.52$, $P < 0.001$). Generality for hawkmoths (5.37 ± 0.85) did not differ significantly from that for hummingbirds (6.64 ± 0.65) and bees (2.96 ± 0.65), but was significantly greater than for general assemblages of pollinators in complete networks (2.59 ± 0.43; $P = 0.028$). Community-level pollinator specialization ($<d'>$) also varied significantly among pollinator
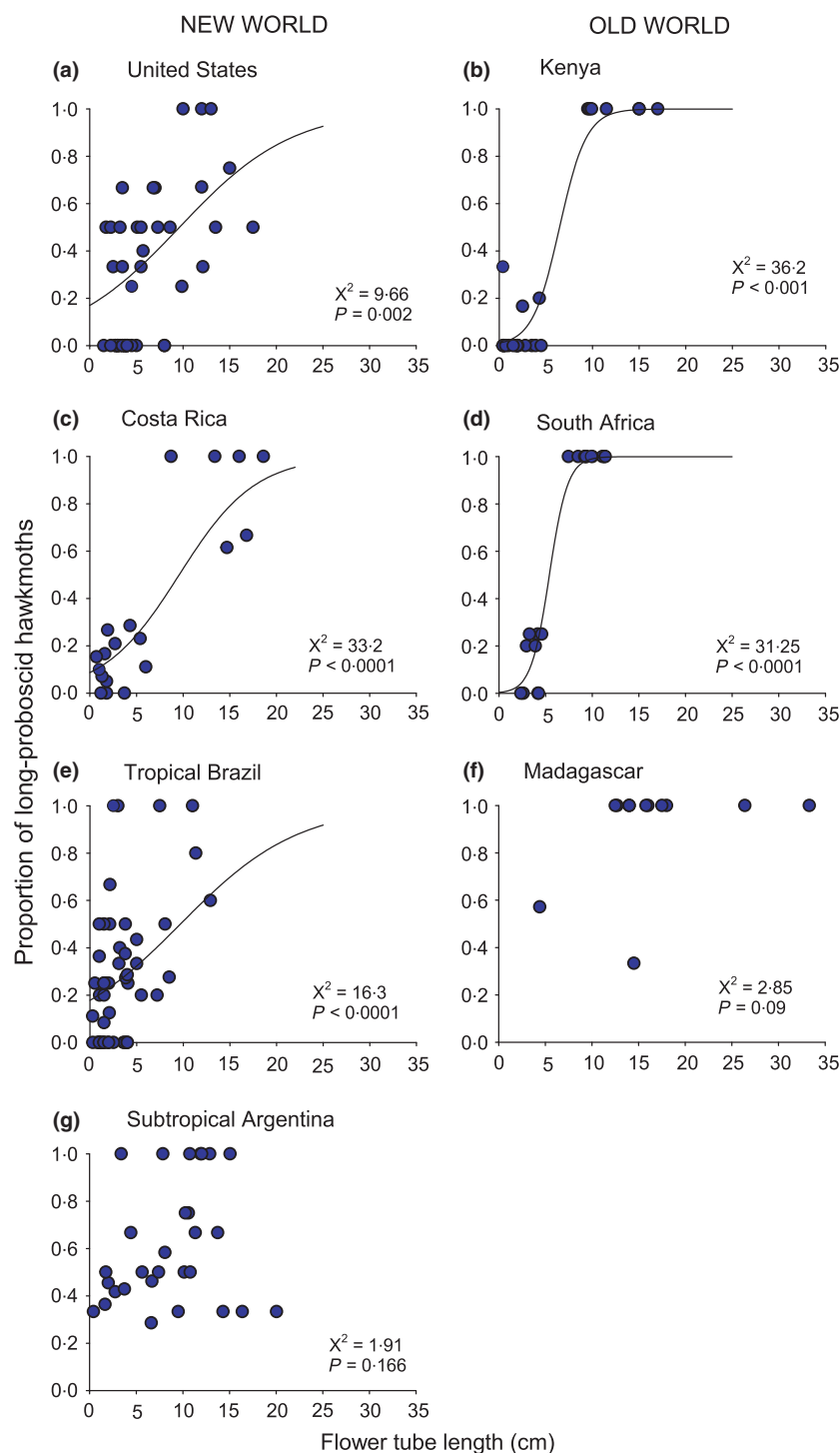
NEW WORLD

OLD WORLD

Fig. 5. The relationships between floral tube length and the proportion of hawkmoths visitors that have long proboscides (>6 cm).

groups ($F_{3,68} = 2.80$, $P = 0.046$), with mean values for hawkmoth–plant networks (0.28 ± 0.05) being significantly lower than those involving bees (0.44 ± 0.35), but not differing from those involving hummingbirds (0.38 ± 0.04) and general assemblages (0.35 ± 0.02). Network-level specialization of hawkmoth species (d_i') did not differ significantly among sites ($F_{7,176} = 0.39$, $P = 0.902$) and was not significantly correlated with proboscis length ($F_{1,176} = 1.49$, $P = 0.222$). There was also no significant interaction between site and proboscis length in terms of

their effect on hawkmoth specialization ($F_{7,176} = 1.65$, $P = 0.124$).

Discussion

Our results show that the frequency distributions of tube length in flowers conforming to the syndrome of hawkmoth pollination are often multimodal, reflecting the availability of two or more hawkmoth pollination niches in most plant communities analysed. While plants show
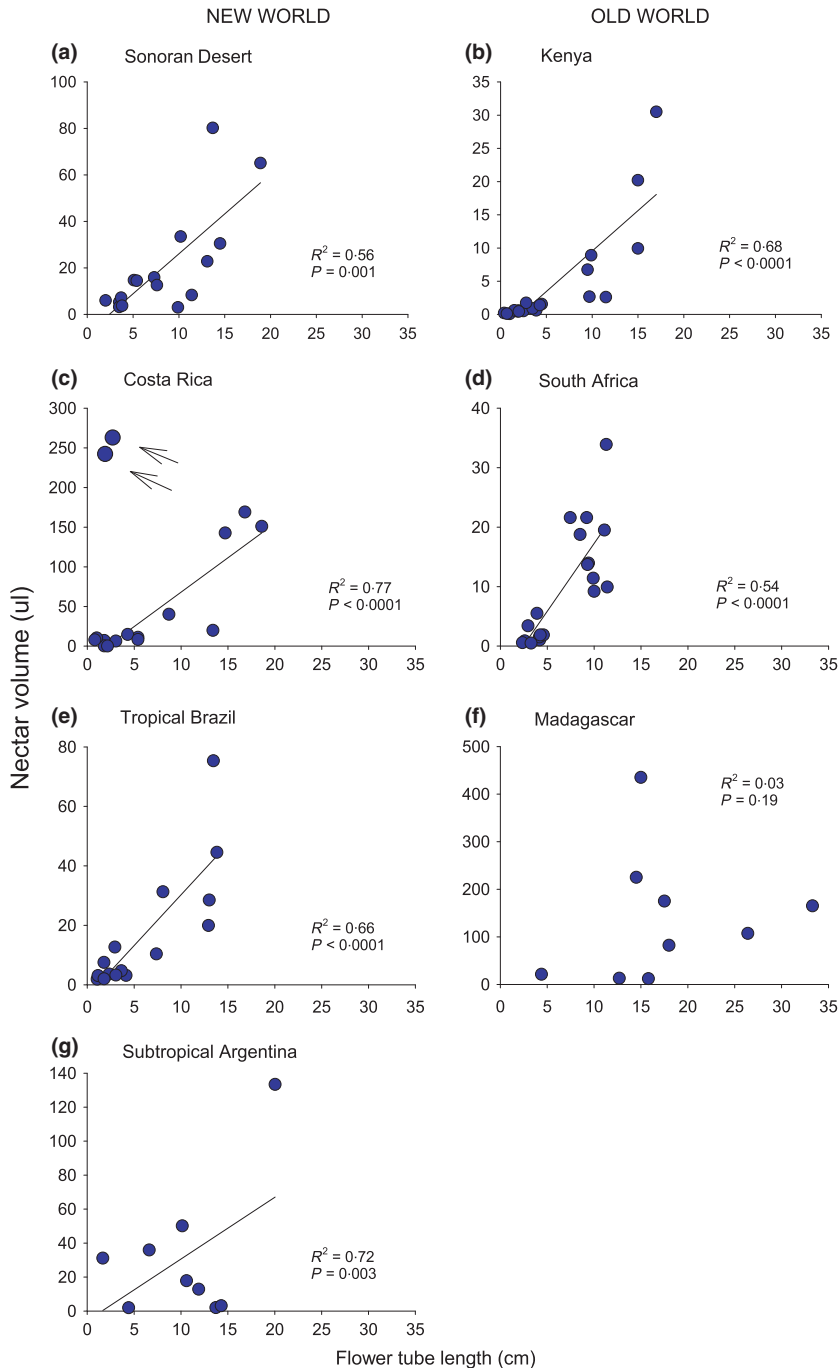
NEW WORLD — OLD WORLD

(a) Sonoran Desert — (b) Kenya — (c) Costa Rica — (d) South Africa — (e) Tropical Brazil — (f) Madagascar — (g) Subtropical Argentina

Nectar volume (ul) / Flower tube length (cm)

$R^2 = 0.56$, $P = 0.001$ (a); $R^2 = 0.68$, $P < 0.0001$ (b); $R^2 = 0.77$, $P < 0.0001$ (c); $R^2 = 0.54$, $P < 0.0001$ (d); $R^2 = 0.66$, $P < 0.0001$ (e); $R^2 = 0.03$, $P = 0.19$ (f); $R^2 = 0.72$, $P = 0.003$ (g)

Fig. 6. The relationships between floral tube length and nectar volume for plant species in various regions of the Old and New World. Two outlying values for species with brush-type flowers (arrowed) were not included in the regression for the Costa Rica site.

increasing specialization for pollination by long-proboscid hawkmoths with increased floral tube length, the opposite pattern is evident for hawkmoths, as longer-proboscid species tend to be more generalist in their foraging habits. This asymmetry, whereby plants specialize on generalist insects for pollination, is well documented for other pollination systems (Vázquez & Aizen 2004).

HAWKMOTH POLLINATOR NICHES FOR PLANTS

A novel aspect of our study is the use of frequency distributions for traits of individuals as well as species as a means of identifying morphometric niches. The rationale is that the availability of a pollination niche is determined not only by the occurrence of a potential pollinator species but also by its abundance. The value in this approach is illustrated by two examples. In North America, studies over more than a century have shown that the assemblage of short-proboscid moths is dominated by *Hyles lineata* and in many habitats this moth is the locally most abundant and effective pollinator for many plant species (Grant 1983) (Table S1). The distribution of proboscis lengths for individual hawkmoths in the Sonoran Desert is a far better match to the distribution of floral tube lengths than is the

distribution of species in which rare and common species have equal weighting (Fig. 3). In the drylands of Africa, the long-proboscid hawkmoth *Agrius convolvuli* is extremely abundant, comprising up to 50% of all hawkmoths in local assemblages (Martins & Johnson 2013; Johnson & Raguso 2016). Several hundred plant species have become adapted for pollination by this moth (Johnson & Raguso 2016), and this is highly likely to be a result of the abundance of individuals.

The assessment of hawkmoth pollinator niches in this study is based largely on light-trapping data. If light trapping is selective, then this method could give misleading results. We have several reasons to be confident that light trapping provides a reasonable measure of the availability of different hawkmoth species in a community. First, the shape of the distribution of hawkmoth traits was largely matched by that for plant traits (Fig. 3). A notable exception was Argentina where the plant species with the longest floral tubes were not matched by very long-proboscid hawkmoths. This may reflect that some long-proboscid hawkmoths at the Argentina site are resistant to light trapping (Butler *et al.* 1999) or, alternatively, that hawkmoths are able to partially enter the floral tubes of Cactaceae, the family that has the longest tubed flowers at the Argentina sites. Secondly, the proboscis length distribution of hawkmoths observed on flowers in Kenya closely matched the proboscis length distribution of hawkmoths light-trapped in nearby Tanzania (Robertson 1977) and in South Africa (Johnson & Raguso 2016). In Madagascar, Nilsson *et al.* (1992) noted that the frequency of hawkmoth species observed on flowers of an orchid species was almost identical to that obtained by light trapping in the same habitat. Beck & Linsenmair (2006) directly tested whether light trapping results in faunistic sample bias in a study of the hawkmoths of north-east Borneo. Their findings suggest that as long as light trapping was continued throughout the night and was repeated for at least a week in each season, it resulted in an accurate, unbiased representation of local sphingid diversity.

GAPS IN TROPICAL ASIA AND AUSTRALIA

Given the information available for the Nearctic, Neotropical and Afrotropical biogeographical realms, the relative dearth of comparable information on hawkmoth pollination in the Australasian and Indomalayan realms is surprising. More than half of the 75 hawkmoth species found in Australia are endemics (Rougerie *et al.* 2014), and this fauna includes many genera (*Agrius, Deilephila, Hippotion, Macroglossum, Nephele* and *Theretra*) that are known to forage for floral nectar elsewhere in Africa and Asia (Nilsson *et al.* 1992; Miyake & Yahara 1998). However, at present, there is very little available information on the range of flowering plants utilized by hawkmoths for nectar in Australia (Hopper 1980; Howell & Prakash 1990; Baum 1995). General syntheses of plant–pollinator affinities in Australia (Armstrong 1979; Williams & Adam 1994;

Hansman 2001), tropical India (Devy & Davidar 2003, 2006) and Malaysia (Momose *et al.* 1998) suggest that sphingophily is at best a very minor pollination niche in these regions.

There are some autoecological studies in Japan that document hawkmoth pollination of specific plants or lineages (Inoue 1986; Miyake & Yahara 1998; Hirota *et al.* 2012, 2013), but lacking for both Japan and neighbouring China are community studies of plant–hawkmoth interactions, such as those we used for this review. The faunistic–ecological disconnect is perhaps strongest in the Oriental or Indomalayan realm, where the hawkmoth fauna is well understood in terms of its diversity, endemism and species distributions (Schulze, Hauser & Maryati 2000; Beck, Kitching & Eduard Linsenmair 2006). Pollen found on light-trapped moths in the Smerinthine tribe Ambulycini confirms that these moths are flower visitors (Beck, Kitching & Eduard Linsenmair 2006). Nevertheless, advanced biogeographical and faunistic knowledge of Indomalayan sphingids translates poorly into ecological representation in community studies of plant–pollinator affinities (Momose *et al.* 1998; Devy & Davidar 2006). One possible reason for this disconnect is the numerical dominance of macroglossine species across this region (202 spp.) as compared with low diversity of sphingine species (24 spp.), with species in the former subfamily thought to prefer disturbed or open habitats omitted from the pollination studies cited above (Beck, Kitching & Eduard Linsenmair 2006).

MORPHOMETRIC TRAITS AND SPECIALIZATION

The trend for longer-proboscid hawkmoths to be generalist in their exploratory foraging seems counterintuitive since the co-evolutionary process that likely gives rise to long proboscides would be expected to involve a certain degree of reciprocal specialization between plants and their pollinators. The conundrum is partially solved by evidence that nectar rewards are greater in longer-tubed flowers than in shorter-tubed ones (Fig. 6). Despite the polyphagous behaviour of long-proboscid hawkmoths, they would stand to gain by modifications in proboscis length that enable them to access nectar from these longer-tubed flowers. The larger rewards in longer-tubed flowers would promote temporal constancy by long-proboscid hawkmoths. Indeed, there is evidence that although long-proboscid hawkmoths are polyphagous, they concentrate their foraging efforts on longer-tubed plant species (Sazatornil *et al.* 2016). Nevertheless, long-proboscid hawkmoths retain the option to visit shorter-tubed flowers for nectar. This behavioural hedge may be important when long-tubed flowers are scarce, for example, during seasonal changes and migration (Haber & Frankie 1989; Amorim, Wyatt & Sazima 2014) or when competition for long-tubed flowers becomes significant. Longer proboscis lengths are correlated with larger body size and lower abundance, so that long-proboscid species may be at a disadvantage in scramble

competition with the more species-rich and individually abundant short-proboscid species (Rodríguez-Gironés & Llandres 2008).

Miller (1997) noted that hawkmoths with the longest tongues tend to utilize larval host plants with inconspicuous or ephemeral growth forms, whereas those with shorter or non-functional tongues tend to utilize more apparent, long-lived plants (shrubs, trees) as hosts. Miller's study revealed that hawkmoths with the longest proboscides also had the largest bodies and did not mature all of their eggs at eclosion, suggesting that adult nectar meals would be needed both to fuel long-distance dispersal for oviposition and to increase fitness by maturing more eggs (O'Brien 1999). One clear prediction from Miller's hypothesis is that moths with long proboscides should demonstrate more generalist foraging patterns, visiting flowers opportunistically, including those that are not primarily adapted to them as pollinators, as they disperse over great distances (Amorim, Wyatt & Sazima 2014). This prediction is consistent with the patterns that emerge from this study.

Data for bumblebees and euglossine bees show a pattern similar to what we found for hawkmoths in that species with longer proboscides visit a greater number of plant species than do those with shorter proboscides (Harder 1985; Borrell 2005). However, hummingbirds show the opposite pattern with longer-billed species showing greater specialization in foraging (Maglianesi *et al.* 2014). The basis for this apparent difference between insects and birds in the relation between mouthpart length and foraging specialization is uncertain. One possibility is that hummingbirds develop better mental maps about the location of plants and are therefore able to focus almost exclusively on long-tubed (and more rewarding) species via targeted 'traplining'. Longer-billed hummingbirds are often local residents, while long-proboscid hawkmoths often travel great distance and have to feed opportunistically.

COMMUNITY-LEVEL SPECIALIZATION

At the community level, our results indicate that hawkmoths are as generalized as hummingbirds and bees in terms of the mean number of plants used for foraging, and even less specialized than bees in terms of ecological specialization ($<d'>$), a measure of discrimination among available plant species. This confirms the findings of earlier studies (Haber & Frankie 1989; Martins & Johnson 2013) that indicate that long-proboscid hawkmoths are polyphagous and visit a wide range of plant species. However, recent analyses of hawkmoth–plant interactions in Argentina and Brazil from a network perspective found that long-proboscid hawkmoths do tend to interact more frequently with plants which have floral tubes that are similar in length to their proboscis (see Sazatornil *et al.* 2016). Very similar results were obtained for the Costa Rica network included in this study (Fig. S3). Therefore, morphological fit between hawkmoths and flowers does appear to

be important for structuring interactions at the community level, even beyond that expected from trait mismatch constraints (Vizentin-Bugoni, Maruyama & Sazima 2014). The most likely reason why long-proboscid hawkmoths tend to show a higher frequency of interactions with longer-tubed species than expected by chance is because they learn to associate floral signals of these species with greater rewards (Balkenius, Kelber & Balkenius 2004; Riffell *et al.* 2008; Kaczorowski *et al.* 2012).

Our data suggest that visitation networks (those based on direct observations) may underestimate the number of interactions involving hawkmoths (Table S3). This is a key difference to other networks in which estimates based on pollen loads usually indicate more specialized interactions than those based on directly observed visitation, something that is usually attributed to flower visitors picking up pollen from only a portion of the species visited (Alarcón 2010; Popic, Wardle & Davila 2013). The reverse pattern for hawkmoths is all the more remarkable given that the number of pollen types was generally greater for the longer-proboscid hawkmoth species. Given that pollen may not be picked up from all short-tubed flowers visited by these moths, the ecological generalization (in the sense of investigating different species) of longer-proboscid hawkmoths may be even greater than suggested by our results. What is certain is that many flower visiting interactions between hawkmoths and plants are unlikely to be observed directly, particularly if they take place in a forest canopy, and that pollen analysis is a key tool for uncovering these interactions. The biggest drawback to pollen analysis is that it is usually difficult to distinguish among the pollen grains of congeneric species. We faced this problem for the analysis of visitors to two species of Cactaceae in Costa Rica and ultimately had to treat these two species as one example of 'Cactaceae' for the community network analysis. Although Old World networks appear more specialized than New World ones, networks in the two regions were sometimes sampled using different methods (mainly direct observations in the Old World, apart from Madagascar where pollen analysis was mainly used, and mainly pollen analysis in the New World). Therefore, more work is needed to confirm whether regional effects on network structure are real or an artefact of methodology.

Although hawkmoths are clearly polyphagous insects, plants clearly adapt to the proboscis length of specific species or guilds of species (Nilsson 1988; Alexandersson & Johnson 2002; Anderson, Alexandersson & Johnson 2010). These guilds are evident as modes in the distributions of proboscis and tube lengths in different regions (Fig. 3). The next step in the analysis of hawkmoth–plant networks will be to investigate whether these guilds correspond to particular modules that exist within these networks (Olesen *et al.* 2007). Such modules may reflect the occurrence of functional niche specialization within hawkmoth–plant networks and perhaps even units of ongoing co-evolution.

The results of the present study suggest that proboscis length will emerge as a key determinant of module formation within hawkmoth–plant networks. However, there are other potential niche dimensions that were not considered in this study, such as the confinement of certain hawkmoths to either open or closed canopy environments (Johnson & Raguso 2016). Even proboscis width (and its match with nectar tube width) has also been shown to provide a dimension of ecological filtration in terms of pollen export and placement (Moré, Sérsic & Cocucci 2007) as well as successful nectar extraction (Coombs & Peter 2010). Scent chemistry is also likely play a role in selective attraction of particular hawkmoths to flowers (Riffell *et al.* 2013), although the generalist exploratory behaviour of hawkmoths would suggest that they have less olfactory specialization than do many other insects, such as oligolectic bees.

In closing, our study has identified a number of critical gaps in our knowledge of hawkmoth–flower interactions. These gaps include (i) a shortage of studies on hawkmoth interactions with short and medium tube length flowers in Madagascar, (ii) an almost complete lack of community-level studies of hawkmoth pollination in Asia and Australia, (iii) a paucity of data on plants pollinated by hawkmoths with the longest proboscides in South America and (iv) a general shortage of direct or video observations to complement indirect records of hawkmoth–plant interactions by means of pollen analyses. A more complete picture of the interactions between hawkmoths and plants globally will give us greater insights into the ecological interdependency of these organisms. Given the rate at which natural habitats in places such as Madagascar and the Neotropics are disappearing, and the already perilous status of some mutualisms between hawkmoths and plants (cf. Suzán, Nabhan & Patten 1994; Gemmill *et al.* 1998; Johnson *et al.* 2004), this baseline information should be gathered as soon as possible and may prove vital for identifying ecological linkages that are required for the preservation of biodiversity.

Acknowledgements

Data accessibility

All data used in this manuscript are present in the manuscript and its supporting information.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. A revision of Grant's (1983) survey of hawkmoth-adapted plants in North America.

Table S2. A hawkmoth–plant network from tropical dry forest in Guanacaste, Costa Rica.

Table S3. Comparison of the visitation, pollen and combined networks from Guanacaste, Costa Rica.

Table S4. Details of the bee only networks used for comparative purposes in this study.

Table S5. Results of Hartigan's diptest for multimodality and AICc values for finite mixture models.

Table S6. Generalized linear models that analyse potential predictors of the number of hawkmoth species, proportion of long-proboscid hawkmoth species and nectar sugar content of flowers.

Fig. S1. The relationships between hawkmoth proboscis length and number of known nectar host plants in six communities.

Fig. S2. The relationships between floral tube length and nectar sugar content for plant species in various regions of the Old and New World.

Fig. S3. Parameter simulations for the Costa Rica network.