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Proboscis curling in a pollinator causes extensive pollen movement and loss

GORDON P. SMITH,[1,3] GOGGY DAVIDOWITZ,[2,3] ROBERT A. RAGUSO[1] and JUDITH L. BRONSTEIN[3] [1]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA, [2]Department of Entomology, University of Arizona, Tucson, AZ, USA and [3]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA

Abstract. 1. Precise pollen placement on floral visitors can improve pollen transfer, but in many plant species, pollen is deposited onto the flexible proboscises of long-tongued insects. These proboscises are curled and uncurled between floral visits, potentially causing pollen to be lost or displaced. Rates of pollen movement and loss resulting from proboscis curling, and hence the potential quality of long-tongued insects as pollinators, are unknown.

2. Here, pollen loss and movement on the proboscises of *Manduca sexta* (Sphingidae) hawkmoths was experimentally measured. It was predicted that (i) proboscis curling causes pollen loss; (ii) pollen that is not lost is displaced from its deposition site; and (iii) repeated curls result in more displacement. Pollen from *Datura wrightii*, an important nectar plant for *M. sexta*, was placed distal to the knee bend on *M. sexta* proboscises, and the number and location of grains was recorded after proboscis curls.

3. Consistent with the hypotheses, proboscis curling caused significant pollen loss. (i) A single curl resulted in the loss of almost 75% of the pollen from the placement site; after repeated curling, 98% of grains were lost from this site. (ii) A single curl was also sufficient to displace pollen across all surfaces of the proboscis, but (iii) further curling did not affect its distribution across surfaces.

4. Together, these results suggest that precise pollen placement on the proboscises of hawkmoths would be unlikely to increase pollen transfer success. Strategies by which flowering plants might mitigate the effects of pollen loss from visitors with flexible pollen-pickup structures are discussed.

Key words. hawkmoths, pollen fate, pollen loss, pollination, proboscis curling.

Introduction

For animal-pollinated plants, precise pollen placement on flower visitors is generally advantageous to reproductive success (Armbruster *et al.*, 2009). The location at which pollen is placed on a pollinator's body can influence the amount of pollen that is lost either passively or through grooming (Tong & Huang, 2017) and can influence whether grains are deposited on conspecific or heterospecific stigmas (Morales & Traveset, 2008; Muchhala & Thomson, 2012; but see Murcia & Feinsinger, 1996). Indeed, in several cases, plants competing for the same pool of visitors have been shown to place pollen at different sites on the pollinator's body. For example, *Burmeistera* spp. place their pollen on different sites on the faces of their nectivorous bat pollinators (*Anoura* spp., Muchhala & Potts, 2007), co-occurring *Pedicularis* species place their pollen on different sites of their shared bumble bee (*Bombus richardsi*) pollinators (Huang & Shi, 2013), and Malagasy orchids place sticky pollinia on different sites on their shared hawkmoth visitor (*Panogena lingens*; Nilsson *et al.*, 1987). This physical separation of pollen on the pollinator's body helps to maintain reproductive barriers between closely related species of plants (Armbruster *et al.*, 1994; Huang & Shi, 2013), reduces the costs associated with pollen transferred to and received from heterospecific flowers, and increases conspecific pollen export (Armbruster *et al.*, 2009).

Precise pollen placement on floral visitors' bodies, however, should translate into benefits for plants only if pollen

Correspondence: Gordon P. Smith, Department of Neurobiology and Behavior, Cornell University, W361 Mudd Hall, Ithaca, NY 14853, USA. E-mail: gosmith2@gmail.com

is unlikely to shift in position after it is deposited on the visitor (Armbruster & Muchhala, 2009). This is unlikely to always be the case. For example, some plant species deposit pollen on flexible structures. Many plants are pollinated by long-tongued Lepidoptera and Diptera and place pollen on their notably flexible proboscises (e.g., Bryant *et al.*, 1991; Johnson & Steiner, 1997). Typically kept coiled or folded during nonfeeding activities, proboscises are straightened using a combination of muscle activity and hydrostatic pressure in response to food cues (Wannenmacher & Wasserthal, 2003; Raguso *et al.*, 2005; Karolyi *et al.*, 2012). After visiting a flower, or group of flowers if they are close enough together, the proboscis is curled or folded until the next floral visit. Because these behaviours lead some surfaces of the proboscises to rub past each other, they have the potential to cause pollen on the proboscis to be lost (Levin & Berube, 1972) or displaced from the site where it was deposited. These phenomena could affect pollen transfer for many plant species: long-tongued insects are common pollinators across many ecosystems (Johnson *et al.*, 2017), and in many cases are the primary or only pollinators of rare or threatened plants (Johnson *et al.*, 2004; Fox *et al.*, 2013). The degree to which proboscis curling causes pollen loss, however, as well as how this might affect plant reproduction, has not to our knowledge been investigated.

Here, we examine experimentally how proboscis curling behaviour affects pollen loss and displacement on the long (~90 mm) proboscis of the hawkmoth *Manduca sexta* (Lepidoptera, Sphingidae). We predicted that (i) proboscis curling will dislodge pollen and cause pollen loss and that more curling will result in more loss. Because coils of the proboscis contact each other, we also predicted that (ii) pollen that is not lost is displaced from its original deposition site. Specifically, because the proboscis curls from the tip, we expected more pollen to be displaced basally than distally. Finally, because each curling event has the potential to displace pollen, we predicted that (iii) repeated curling leads to greater displacement.

Methods

Moth rearing and greenhouse plants

Flower-naïve adult *M. sexta* were obtained from a laboratory colony at Cornell University (Broadhead *et al.*, 2017). Larvae in this colony were reared on a well-established artificial diet (Bell & Joachim, 1976) in which cornmeal was substituted for wheat germ (Goyret *et al.*, 2009). Larvae were a long-day cycle to stimulate a shorter pupation time (LD 16:8 h**;** 24 °C; 40–50% RH). Three days prior to eclosion, pupae were separated from the colony and allowed to eclose in polypropylene mesh cages (31 × 31 x 32cm). Moths were not manipulated until approximately 12 h posteclosion to ensure that their wings had completely dried. Both male and female moths were used; we detected no statistically significant differences between the sexes in pollen displacement (unpublished data).

We used the anthers of *Datura wrightii* (Solanaceae) as a source of ecologically relevant pollen. In the Southwestern United States, *M. sexta* frequently visits flowers of *D. wrightii* for nectar and is the primary pollinator of this plant (Alarcón *et al.*, 2008; Bronstein *et al.*, 2009). The funnel-shaped flowers of *D. wrightii* are among the largest in the North American flora, so large that hovering *M. sexta* moths often land within them while probing for nectar (Raguso *et al.*, 2003). Although the moth wings and other body parts may contact the reproductive structures of *D. wrightii* flowers when the moths have fully entered the flowers, their first contact with the anthers is with the proboscis while probing and approaching the flowers (pers. obs.). As a result, wild *M. sexta* moths carry large amounts of *D. wrightii* pollen on their proboscises (Alarcón *et al.*, 2008). *D. wrightii* plants were grown from seed gathered at the Santa Rita Experimental Range, Santa Cruz Co., AZ, U.S.A. Plants were grown in a Sun-Gro Metro-Mix 360 at day/night temperatures of 24 °C/21 °C. Anthers were gently dissected out of newly opened flowers to ensure that pollen was not accidentally dislodged prior to their use.

Laboratory experiment

Moths were restrained in a 15 ml Falcon tube whose tip had been removed, allowing only the head to extend from the tip. The proboscis was then unrolled and held in place using soft forceps that were themselves held by clamps (Fig. 1a), allowing precise placement of pollen onto the proboscis without risk of movement or injury to the restrained moth. For each moth, a fresh *D. wrightii* anther was gently tapped once on the dorsal surface of the proboscis distal to the articulated "knee bend" (Fig. 1; see Krenn & Mühlberger, 2002). This surface frequently contacts *Datura* anthers during natural foraging (pers. obs.). The anther was held horizontally during this tap, such that its widest surface would contact the proboscis and extra pollen would not be smeared onto the proboscis through lateral movement.

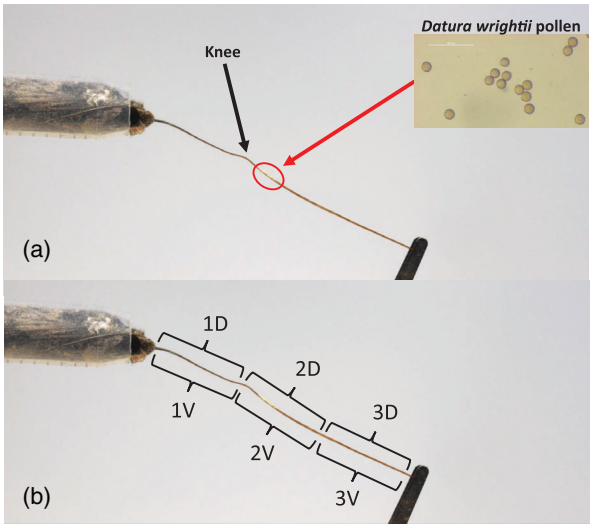

Fig. 1. Method for applying pollen to the proboscises of *Manduca sexta* hawkmoths. (a) *Datura wrightii* pollen was applied to the dorsal surface of the proboscis distal to the knee. (b) Representation of the six sites we distinguished when examining pollen movement. 1D: Dorsal-proximal site; 2D: Dorsal-central; 3D: Dorsal-distal; 1V: Ventral-proximal; 2V: Ventral-central; 3V: Ventral-distal.

After the pollen had been applied, moths were randomly assigned to one of three treatments ($N = 10$ for each): no curl, one-curl, and 24 h. *No-curl moths:* the moth, still in the restraining apparatus, was transferred to a −20 °C freezer, with the proboscis still held extended by the clamped forceps. After 24 h in the freezer, the pollen on the proboscis was removed by rubbing cubes of fuchsin-dyed glycerin gel (Kearns & Inouye, 1993) along different dorsal and ventral surfaces of the proboscis. The pollen adhered to the cube, which was then melted on a clean microscope slide for the pollen grains to be counted. Pollen was separately collected from six sites: the dorsal and ventral surfaces for each of the proximal, central and distal thirds of the proboscis (see Fig. 1b). Subsequently, we refer to the different longitudinal segments of the proboscis (proximal, central and distal) as 'segments', the collection side (dorsal and ventral) as 'surfaces', and their combinations as 'sites'. Using this terminology, pollen was initially placed on the dorsal-central site.

One-curl moths. After the deposition of pollen, the proboscis was released; as it was not feeding, the moth curled its proboscis naturally. Once the proboscis was fully curled, the moth was placed in a −20 °C freezer. To keep the movement of the moths consistent among treatments, they were left within the Falcon tubes. After 24 h, each moth was removed from the freezer and the Falcon tube was secured to a dissecting board such that the moth's ventral surface was facing upwards. The labial palps were removed to allow access to the proboscis, then the proboscis was carefully unrolled from its base using an insect pin to approximately 1/3 of its length. While this unrolling may move pollen within this 1/3 (specifically its ventral surface), stopping after each 1/3 prevented pollen movement between segments or sites. The partially unrolled proboscis was prevented from rerolling using insect pins but remained far enough above the dissecting board that the dorsal surface of the proboscis did not contact the board and could still be accessed. Separate fuchsin gel cubes were then rubbed along the ventral and dorsal surfaces of the exposed section, as well as the surfaces of any insect pins that had contacted those sites during uncurling. All gel cubes were then melted onto clean microscope slides. All tools were cleaned with alcohol swabs between the sampling of each site to prevent any pollen transfer during the pollen collection. This process was repeated twice more, with the proboscis being unfurled further with each step, for the second and third segments to generate slides of all six sites described above.

24-h moths. The proboscis was released after pollen placement and allowed to curl, after which the moths were removed from the Falcon tube and placed for 24 h in groups of 2–3 in polypropylene mesh cages (61 × 61 × 91 cm), which provided sufficient room for the moths to fly freely. These cages also contained a nonflowering *Oenothera harringtonii* plant and a nectar source that the moths could visit. The nectar source was an artificial flower, a white funnel attached to a small tube containing a 20% sucrose solution. To prevent the sugar solution from washing the pollen off the proboscis, artificial nectaries were designed such that only the tip of a proboscis would be able to reach the sugar solution. The moths were allowed to forage freely for 24 h. Although they were not continuously observed to count the number of proboscis curls, moths were seen to visit the artificial flower during this period (for videos, see Video S1), and artificial nectar volumes decreased; we, therefore, inferred that moths within this treatment curled and uncurled their proboscises multiple times. Moths were then removed from the cage, returned to the Falcon tubes, and frozen at −20 °C. The moths were removed from the freezer after 24 h and processed using the same procedure as the one-curl moths. For two individuals, the *Oenothera* plant in their foraging arena produced a flower during the treatment period. As the proboscises of these moths may have contacted *Oenothera* anthers or stigmas, these two individuals were excluded from all analyses.

Data analysis

All statistical analyses were performed using R version 3.6.3 (R Development Core Team, 2014). *Prediction 1*: To determine the total amount of pollen loss from proboscises attributable to curling, we used a Poisson generalized linear model (GLM) to compare total grain number (across all proboscis sites) among treatments. *Prediction 2*: To determine the degree to which pollen moved among proboscis placement sites, we compared the number of pollen grains at each site using a Poisson generalized linear mixed model (GLMM). In this model, treatment, proboscis segment, proboscis surface, and their interactions were fixed effects, and moth individual was a random effect. To determine whether more pollen moved basally than distally from the initial deposition site, we specifically examined the treatment by segment interaction comparing the first and third segments for the one-curl and 24-h treatments. *Prediction 3*: To determine whether more curling caused more pollen displacement, we performed planned post hoc comparisons by rerunning the models described above with only the one-curl and 24-h treatments. For each model, alpha values were adjusted using a false rate discovery correction.

Results

Prediction 1: curling causes pollen loss. The total number of pollen grains on the moths' proboscises differed among the treatments. Consistent with the prediction, one-curl moths carried significantly fewer grains than no-curl moths (Poisson GLM; see Table 1a, Fig. 2), and 24-h moths carried fewer grains than did either one-curl moths or no-curl moths (no curl: mean 770 ± 88.3 grains; one-curl: mean 484.2 ± 73.4 grains; 24-h: mean 60.9 ± 28.6 grains).

Prediction 2: curling causes pollen displacement. Consistent with our prediction, the distribution of pollen grains across the proboscis sites also differed among treatments (Fig. 3). The central proboscis segment, where pollen was initially deposited, held significantly more pollen at the end of the experiment than did the basal and distal segments (Poisson GLMM, see

Table 1. Model terms and output for the overall amount of *Datura wrightii* pollen on *Manduca sexta* proboscises. An * in the fixed effects column indicates that the model includes the listed fixed term as well as its interactions with other fixed terms.

	Model	Output				
(a)	Fixed effects		Estimate	Std.Error	*z* value	Pr (>|z|)
	Pollen∼	(Intercept)	6.65	0.01	583.22	**0.00**
	Treatment	One curl	−0.46	0.02	−25.29	**0.00**
		24 h	−2.54	0.05	−54.31	**0.00**

(b)	Fixed effects		Value	Std.Error	DF	*t*-value	*P*-value
	Pollen∼	(Intercept)	−1.648	3.063	125	−0.538	0.592
	Treatment	One curl	4.772	3.079	25	1.550	0.134
	*Segment	24 h	3.198	3.140	25	1.018	0.318
	*Surface	Segment 2	8.099	3.061	125	2.646	**0.009**
		Segment 3	1.705	3.327	125	0.512	0.609
		Surface V	1.609	3.352	125	0.480	0.632
		One curl:Segment 2	−6.113	3.075	125	−1.988	**0.049**
		24 h:Segment 2	−7.164	3.160	125	−2.267	**0.025**
		One curl:Segment 3	−1.200	3.345	125	−0.359	0.721
		24 h:Segment 3	−1.977	3.478	125	−0.568	0.571
		One curl:Surface V	−0.210	3.367	125	−0.062	0.950
		24 h:Surface V	−0.357	3.437	125	−0.104	0.918
		Segment 2:Surface V	−3.483	3.356	125	−1.038	0.301
		Segment 3:Surface V	0.423	3.628	125	0.117	0.907
		One curl:Segment 2:Surface V	1.434	3.374	125	0.425	0.672
		24 h:Segment 2:Surface V	1.976	3.498	125	0.565	0.573
		One curl:Segment 3:Surface V	−1.537	3.653	125	−0.421	0.675
		24 h:Segment 3:Surface V	−0.811	3.817	125	−0.213	0.832
	Random effects		Intercept	Residual			
	∼1 | Moth ID	StdDev:	0.409	4.089			

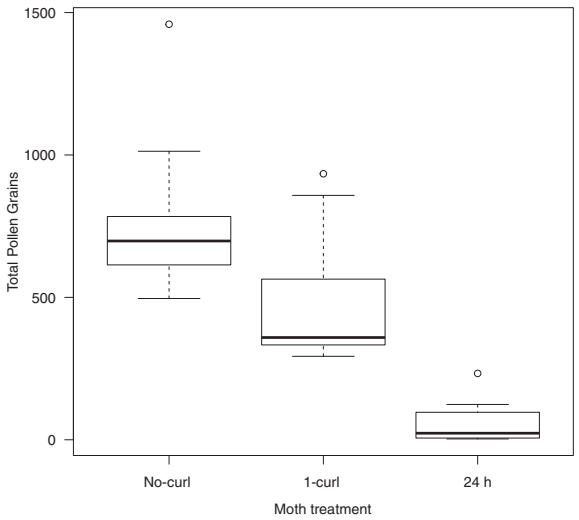

Fig. 2. Total *Datura wrightii* pollen held on *Manduca sexta* moth proboscises after each treatment. The thick black lines represent the mean, with the box bounding the first and third quantiles and the whiskers representing the first and fourth quantiles.

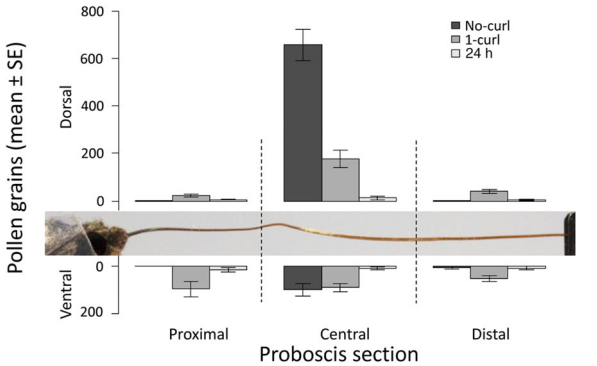

Fig. 3. The distribution of pollen on the proboscises of *Manduca sexta* hawkmoths in the no curl (dark grey), one curl (medium grey) and 24 h (light grey) treatments. The location of each bar corresponds to the site from which pollen was collected for that bar.

Table 1b). Segment interacted with treatment, such that no-curl moths had significantly more pollen grains on the central segment than did one-curl or 24-h moths. No-curl moths had more pollen grains at the site of deposition than the other treatments (dorsal-central; no-curl mean: 658.2 ± 66.8; one-curl mean: 176.3 ± 36.4; 24 h mean: 13.4 ± 7.9), and the majority of pollen on no-curl moths was found at this site (85%). Of the remaining pollen on no-curl moths, 13% was on the ventral-central site, and only ∼2% of pollen was found on the basal or distal segments. In contrast, the grains on one-curl and 24-h moths were more evenly spread across all the sites, such that dorsal-central pollen accounted for only 36% and 22% of remaining pollen, respectively. Across treatments, the amount of pollen present at the end of the experiment was not significantly different between the first and third segments,

Table 2. Model terms and output comparing the amount and location of *Datura wrightii* pollen held on one-curl and 24-h treatment *Manduca sexta* moths. An * in the fixed effects column indicates that the model includes the listed fixed term as well as its interactions with other fixed terms.

	Model	Output				
(a)	Fixed effects		Estimate	Std.Error	*z* value	Pr (>|z|)
	Pollen∼	(Intercept)	6.18	0.01	403.21	**0.00**
	Treatment	24 h	−2.07	0.05	−43.62	**0.00**

(b)	Fixed effects		Value	Std.Error	DF	*t*-value	*P* value
	Pollen∼	(Intercept)	3.116	0.344	80	9.061	**0.000**
	Treatment	24 h	−1.640	0.807	16	−2.031	0.184
	*Segment	Segment 2	1.986	0.309	80	6.433	**0.000**
	*Surface	Segment 3	0.505	0.367	80	1.378	0.295
		Surface V	1.400	0.323	80	4.330	**0.000**
		24 h:Segment 2	−1.051	0.876	80	−1.199	0.351
		24 h:Segment 3	−0.777	1.118	80	−0.695	0.652
		24 h:Surface V	−0.147	0.852	80	−0.172	0.864
		Segment 2:Surface V	−2.049	0.371	80	−5.516	**0.000**
		Segment 3:Surface V	−1.113	0.439	80	−2.534	**0.032**
		24 h:Segment 2:Surface V	0.542	1.092	80	0.496	0.677
		24 h:Segment 3:Surface V	0.725	1.309	80	0.554	0.677
	Random effects		Intercept	Residual			
	∼1 | Moth ID	SD	0.549	4.246			

All *P* values were adjusted using the false rate discovery method. (a) Total pollen held on the proboscis across all sites. (b) Pollen held on each proboscis site.

nor was there a significant interaction with treatment in either segment. Similarly, there was no statistically significant effect of proboscis surface (dorsal vs. ventral) or interaction between surface and treatment.

Prediction 3: more curling causes more pollen displacement. Contrary to our predictions, the distribution of pollen across sites did not differ between one-curl and 24-h moths (see Table 2). Consistent with the full model described above, in this model including only the curling treatments 24-h moths carried significantly less pollen than one-curl moths, and proboscis segment 2 held significantly more pollen than other segments. Additionally, in this model, the ventral surface of the proboscis held significantly more pollen than the dorsal surface; the surface also interacted with segments such that the ventral surfaces of segments 2 and 3 had less pollen than the dorsal surfaces of those segments. Importantly, however, there was no main effect or significant interaction including treatment on the number of pollen grains found.

Discussion

Although all pollinators are expected to lose pollen (see Inouye *et al.*, 1994), the rate and frequency of pollen loss from pollinators' bodies, and how specific behaviours affect these rates, have rarely been examined directly. We predicted that proboscis curling behaviour in long-tongued Lepidoptera, important pollinators for plant taxa worldwide (Johnson *et al.*, 2016), would cause substantial pollen loss and displacement. Consistent with our predictions, proboscis curling by *M. sexta* led to substantial pollen loss and movement. Compared with moths that were not allowed to curl their proboscises, moths that curled their proboscis once carried ∼40% less pollen overall, and 74% less pollen at the site where grains were originally deposited. Additional curling behaviour caused further losses: moths allowed to forage for 24 h after experimental pollen deposition carried less than 8% of the total pollen grains carried by moths prevented from curling the proboscis and less than 2% of the grains were present at the site where it was deposited.

A substantial amount of pollen was transferred to other sites on the moths' proboscises in the curling treatments (∼64% of remaining pollen in one-curl moths, ∼78% in 24-h moths), but not in the no-curl treatment, suggesting that the movement was not simply due to jostling during the experimental manipulation. The one exception to this was the pollen present on the central-ventral site in no-curl moths (mean 101 grains); this pollen likely fell through the lateral hairs lining the proboscis. In the one-curl and 24-h treatments, more pollen was found on the ventral surfaces of moths' basal and central segments, which were the most likely to contact the dorsal-central deposition site during curling. Importantly, a single curling event was sufficient to spread pollen across the entire proboscis; further curling did not significantly change its relative distribution. This suggests that while pollen loss from all sites on the proboscis continued with additional curling, transfer among sites may primarily occur during the first curl when the largest amount of loose pollen is available to be transferred. As *M. sexta* moths do not actively groom themselves of pollen, this loss and movement is the result of passive loss (Inouye *et al.*, 1994) attributable to the curling itself. Although we did not directly assess the fate of the pollen that was lost, the majority was likely either shed from the proboscis entirely or transferred into the facial cavity, where it would be unavailable for stigma contact (Courtney *et al.*, 1982).

Given the rarity of studies directly quantifying pollen loss from pollinator bodies, how the loss we observed compares

to other pollinator groups is not immediately clear. More commonly, studies have measured pollen carry-over, the number of pollen grains deposited on successive stigmas during a foraging bout. In these studies, the amount of pollen deposited decreases relatively little with each successive floral visit in hummingbirds (Price & Waser, 1982), bees (Thomson, 1986) and bats (Muchhala & Thomson, 2012). Floral visitors often deposit only a very small fraction of the pollen that they remove during a single visit (e.g., 0.6% in bumblebees on *Erythronium;* Harder & Thomson, 1989). Whether the rest of that removed pollen remains on the visitor or is lost (either passively or via grooming) is often unclear. One exception comes from a study of *Bombus terrestris*, in which 93% of *Echium vulgare* pollen was found to have remained on the bee after it flew to a second flower, with ∼6% of grains lost to grooming and the remaining ∼1% lost passively (Rademaker *et al.*, 1997). This rate of passive pollen loss is substantially lower than we found for pollen on moth proboscises (∼40% after one curl); studies in other pollinators would be necessary to test whether loss rates for Lepidoptera are consistently higher than other groups.

To our knowledge, pollen loss from pollinators' bodies over longer time periods has only directly been quantified in laboratory environments with Lepidoptera, and the reported rates of loss vary considerably. Small tortoiseshell butterflies (*Aglais urticae*) lost *Petasites hybridus* pollen from their heads gradually over approximately 4 days while visiting other flowers (Courtney *et al.*, 1982), and *Helicoverpa armigera* moths lost *Brassica napus* pollen from their proboscises gradually over several days while restrained (Richards *et al.*, 2005). In contrast, *H. armigera* lost 80% of the *Gossypium hirsutum* pollen they carried in only 8 h (Richards *et al.*, 2005), and *Colias eurytheme* lost between 15% and 52% of the *Phlox* spp. pollen after their first proboscis curl (Levin & Berube, 1972). *H. armigera* lost 50% of *Helianthus annuus* pollen they carried after 24 h; loss increased to 98% if those 24 h were spent in the presence of a different species of flower (Socorro & Gregg, 2001). These rates of pollen loss are similar to the overall loss rates quantified in our study (∼40% after one curl, ∼92% after 24 h); however, none of these studies examined the possibility that pollen had shifted placement on the pollinator body. Variation in loss rate is likely due to a combination of differences in pollen placement location (e.g., eyes vs. proboscis), proboscis length (which could influence the amount of overlap and contact between proboscis sites), and pollen characteristics (e.g., size, shape and stickiness). Disentangling these variables and determining how rates of pollen carry-over in these species compare with non-lepidopteran pollinators would be a valuable avenue for future work.

Potential plant consequences

Our results suggest that proboscis curling causes pollen loss and that the precise pollen placement mechanisms that improve conspecific pollen transfer in other plants (Muchhala & Potts, 2007; Huang & Shi, 2013) are unlikely to be effective for flowers visited by pollinators with long flexible proboscises. Certain plant traits, however, have the potential to reduce pollen loss associated with proboscis curling. For example, moths often leave their proboscises unfurled between visits if flowers are close enough together (pers. obs.); plants that produce inflorescences or grow in very dense patches may avoid curling-associated loss for some transfers. This may, however, increase the rate of geitonogamy (fertilization by pollen from a different flower on the same plant), as the moths leave their tongues unfurled between nearby flowers but curl them as they move to more distant flowers or patches. Many plants visited by hawkmoths also employ mechanisms that make pollen more likely to adhere to the proboscis, such as sticky pollinia (Nilsson, 1983; Johnson & Steiner, 1997) or multiple pollen grains bound together with viscin threads or sticky compounds (Hesse, 1981), as seen, for example, in hawkmoth-pollinated *Oenothera* spp. (Gregory, 1963) and *Mandevilla* spp. (Moré *et al.*, 2007). While the relative stickiness of *Datura* pollen has not been examined to our knowledge, its pollenkitt (the lipid-rich surface of the pollen grain) may share some of these properties.

It is worth noting, however, that spreading pollen across the proboscis may actually benefit plants in some cases, as it effectively increases the size of the pollen transfer surface and therefore the probability that at least some successful transfer occurs. This may be especially beneficial for pollen-limited plants or flowers in which visitor approach angles are variable (e.g., *D. wrightii*; pers. obs.). Pollen transferred to the facial cavity could also be transferred back to the proboscis after some time, potentially increasing the probability of long-distance pollen transfer. Whether the pollen would remain viable after this process, however, is unclear (Dunwell & Sunderland, 1976).

Whether our results are relevant to other plants that present their pollen as loose grains is therefore unclear. We believe, however, that our results are generalizable: in a parallel experiment using pistachio (*Pistachia vera*) pollen, we found patterns of pollen loss and displacement nearly identical to those reported above for *Datura* (Video S2). *P. vera* pollen is wind dispersed and has a very thin pollenkitt (Bahramabadi *et al.*, 2018) that is presumably less sticky than that of *D. wrightii*. Thus, these results may suggest that the dynamics we found are due to the biophysics of pollen-sized grains on proboscises, rather than the result of pollenkitt properties.

Finally, although many plants pollinated by Lepidoptera place pollen on the proboscis (e.g., Jennersten, 1984; Bryant *et al.*, 1991; Socorro & Gregg, 2001), other plants place pollen on structures such as eyes (Johnson and Bond, 1994; Maad & Nilsson, 2004), wings (Cruden & Hermann-Parker, 1979; Murphy *et al.*, 1984) or thorax (Murphy, 1984; Alarcón *et al.*, 2008), bypassing potential loss from proboscis movement. From the plant's perspective, traits and strategies such as sticky pollen and nonproboscis pollen placement sites could improve the rate of pollen transfer by moths. Investigating how these traits influence pollination success by long-tongued floral visitors, as well as whether these traits are overrepresented in moth-pollinated flowers compared with flowers pollinated by other groups, is an important step to understanding pollen transfer efficiencies by flexible structures.

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Data availability statement

All data associated with this study is archived on Dryad under the title of this article.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Model terms and output for the overall amount of Pistachio pollen on moth proboscises.

Table S2. Model terms and output comparing the amount and location of Pistachio pollen held on one-curl and 24-h treatment moths. All *P* values were adjusted using the FDR method. (a) Total pollen held on the proboscis across all sites. (b) Pollen held on each proboscis site. FDR, false rate discovery

Figure S1. Total *Pistachia vera* pollen held on moth proboscises. The thick black lines represent the mean, with the box bounding the first and third quantiles.

Figure S2. The distribution of *Pistachia vera* pollen on the proboscises of *Manduca sexta* hawkmoths following 0 curling events (no curl, dark grey), one curling event (one-curl, medium grey), or 24 h of foraging on artificial flowers (24 h; light grey). The location of each bar corresponds to the site from which pollen was collected for that bar. Pollen was initially placed on the dorsal-central site of the proboscis; pollen was not collected from other sites in no-curl moths, as the pollen was collected immediately after it was placed.

Video S2. A brief description of the methods and results found in a parallel study using the pollen of pistachio (*Pistachia vera*). This parallel study used methods only slightly modified from those described in the main text, and the pollen displacement patterns we observed were nearly identical to those presented for *Datura wrightii*.

Video S1. Videos showing moths in the 24-h treatment visiting the artificial nectary after pollen had been applied to their proboscises.

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