

# Facial area and hairiness of pollinators visiting semi-natural grassland wild plants predict their facial pollen load

JÉRÉMIE GOULNIK,<sup>1</sup> SYLVAIN PLANTUREUX,<sup>1</sup> COLIN VAN REETH,<sup>2</sup> MATHILDE BAUDE,<sup>3</sup> GEOFFREY MESBAHI<sup>1,4</sup> 

and ALICE MICHELOT - ANTALIK<sup>1</sup>  <sup>1</sup>LAE, Université de Lorraine, INRAE, Nancy, F-54000, France, <sup>2</sup>Centre de Recherches sur les Ecosystèmes d'Altitude (CREA Mont-Blanc), 67 lacets du Belvédère, Chamonix Mont-Blanc, 74400, France, <sup>3</sup>LBLGC EA 1207, Université d'Orléans, INRAE USC 1328, Orléans, 45067, France and <sup>4</sup>Parc Naturel Régional des Vosges du Nord, La Petite Pierre, 67290, France

**Abstract.** 1. Consequences of a decline in pollination function in semi-natural ecosystems are largely unknown due to variability in pollinator effectiveness, that is, their contribution to pollen deposition alone. While pollination effectiveness has been shown to be related to body size and hairiness of pollinators for some crops, studies encompassing a wide diversity of pollinators interacting with wild plant communities are lacking.

2. Thus, the relationships between pollen load, which is a measurement of pollen transport ability and a proxy of pollinator effectiveness, and morphological traits of pollinators sampled in 16 grasslands in Moselle, France, were investigated. The area, hairiness, and pollen load of each pollinator's face were measured for 658 individuals from 127 bee and fly species interacting with 36 wild plant species. Pollinator dry mass was also measured on 543 individuals from 109 species.

3. Dry body mass and facial area of pollinators were positively linked. This study highlights that bees transported significantly more pollen grains on their face than flies. Furthermore, bees' faces were larger and hairier. We also found that pollinators' facial pollen load increased with facial area and hairiness when we considered all pollinators. However, hairiness is not significant within pollinator group (bees or flies), mirroring a potential phylogenetic signal.

4. Hence, this study shows a wide diversity of pollinator and plant species in which larger and hairier pollinators may transport more pollen grains, at least on their face. However, future studies involving other pollinator body parts are needed to generalise these relationships.

**Key words.** Bees, Diptera, effect trait, functional trait, insect, pollination function.

## Introduction

Pollinators are declining worldwide mainly due to agriculture intensification and habitat loss (Potts *et al.*, 2010; Roulston & Goodell, 2011; Hallmann *et al.*, 2017), while 87.5% of flowering plant species worldwide depend on them to ensure their sexual reproduction (Ollerton *et al.*, 2011). However, knowledge about pollinator decline differs among taxa (Hallmann

*et al.*, 2017; Møller, 2020). Decline is well documented for bees but remains incomplete for other taxa (e.g. Diptera). Yet, pollinator taxa seem unequally susceptible to environmental disturbances. For instance, flies may respond differently from bees to agriculture intensification in grasslands (Kühnel & Blüthgen, 2015).

Pollinators do not have equivalent pollination effectiveness (reviewed by Willcox *et al.*, 2017), that is, the ability to transport and deposit pollen during plant–pollinator interaction. Hence, predicting the consequences of pollinator declines or shifts in community composition on the pollination function

Correspondence: Jérémie Goulnik, Université de Lorraine, INRAE, LAE, F-54000 Nancy, France. E-mail: jeremiegoulnik@gmail.com

(i.e. transfer of pollen from flower stamens to stigmas) requires assessing the pollination effectiveness of insect species.

At the community scale, pollination function is usually studied in two main ways. Pollination function can be simply approximated by plant–pollinator interaction frequencies or more accurately by estimating pollen transfer itself (Bartholomé & Lavorel, 2019). Pollinator pollen load and the amount of pollen deposited per interaction provide more information about pollinator effectiveness than interaction frequency alone (King *et al.*, 2013). However, differences in pollen transfer among pollinators have yet to be well established in the literature. These differences may be explained by pollinators' effect traits, that is, functional traits (*sensu*; Violle *et al.*, 2007), which determine the effects of organisms on ecosystem function (Lavorel & Garnier, 2002). Effect traits must be considered to understand and predict relationships between the diversity and composition of pollinators visiting flowers and pollination function in communities in natural habitats, especially following human disturbance (Lavorel *et al.*, 2013).

Recent studies have highlighted the potential role of body size and hairiness as effect traits due to their positive relationship with the amount of pollen transported and deposited per interaction (Stavert *et al.*, 2016; Bartomeus *et al.*, 2018; Phillips *et al.*, 2018). Furthermore, the number of pollen grains transported may also reflect plant adaptation to the traits of their main pollinator. Indeed, a larger pollinator exchange area implies a lower probability of pollen deposition on the stigma. Following this hypothesis, Cruden (2000) suggested that flowers visited mainly by larger pollinators would produce more pollen grains than those visited by smaller pollinators. He did not consider pollinator hairiness, however, which could modify the influence of pollinator exchange area because more hair per unit area could pick up and transport more pollen grains.

Studies examining pollinator effect traits considered only a few plant species, which were often cultivated plants with roughly the same flower morphology (Stavert *et al.*, 2016; Bartomeus *et al.*, 2018; Phillips *et al.*, 2018). Furthermore, these studies focused on relatively few pollinator species, with a usual bias towards bees. Hence, these pollinator effect traits still need to be validated for wild plant species and for a larger pool of them. Comparing pollinator effectiveness among pollinator groups may be misleading because plant species differ in pollen limitation and morphology (e.g. Harder & Barrett, 1996; Larson & Barrett, 2000). Consequently, investigating effect traits related to pollination effectiveness of many pollinator species interacting with many plant species may not allow for direct predictions of pollinator effectiveness of pollinator groups (e.g. flies vs. bees) due to large differences in matching trait values.

We studied a set of 127 pollinators and 36 plant species found in semi-natural grasslands in France. Our objective was to reveal differences in pollen transport by pollinators that could be explained by differences in morphological effect traits. In our study, we considered only the face of pollinators because this body part, particularly the space between the antennae, has been observed to be a likely 'safe site' (i.e. a part of the body difficult to groom), at least for some *Bombus terrestris* and *Apis mellifera* (Koch *et al.*, 2017). Furthermore, the face of pollinators is a main body part for pollen transport (Phillips

*et al.*, 2018), and its hairiness has been related to total pollen load and single-visit deposition by Stavert *et al.* (2016). By doing so, we could relate facial area and hairiness directly to the number of pollen grains present on it while limiting sampling time per pollinator individual. Hence, we hypothesized that the relation between pollen load and pollinator effect traits is well estimated when focusing on pollinators' faces.

In particular, we examined the two following questions: Is pollinator facial pollen load positively related to pollinator facial area and hairiness? Can we explain these relationships by pollen production (i.e. number of pollen grains produced per floral unit) of plant species? We hypothesized that pollinator facial pollen load increases with pollinator facial area and hairiness but that this increase also reflects an adaptation of plant species to increase pollen dispersal with enlargement of the exchange area (i.e. pollinator facial area in this study).

## Material and methods

### Study sites

This study was performed in 16 semi-natural grasslands in Moselle, Northeastern France, in a 12-km<sup>2</sup> circle centred on Sarrebourg (48°73 N, 7°05E, 250 m a.s.l.). The climate of our study site is semi-continental and has a wide range of temperature between winter and summer. From April to July 2017, mean daily temperature was 15.3°C, and cumulative precipitation was 291 mm. The landscape context is characterised by semi-natural habitats ranging from 32 to 70% of the area in a 1000-m radius around each grassland, and grasslands belong to a local gradient of land use intensification. Details of grassland characteristics are given in Goulnik *et al.* (submitted).

### Pollinator sampling and preparation

A single operator (J.G.) sampled pollinators from May to August 2017 with a sweep net on one 400-m<sup>2</sup> (100 m long × 4 m wide) transect per grassland. Insects clearly in contact with reproductive parts of plants were sampled and considered pollinators. The plant species with which each pollinator had interacted was noted. Pollinators were sampled 3–5 times per grassland throughout the season, from 10:00 to 18:00 on sunny days without wind and with temperature above 18°C. Each time, pollinators were sampled for 15 min per grassland, stopping the stopwatch during the time each pollinator was caught and manipulated. In the field, captured pollinators were placed in a clean vial and stored at 4°C in a cooler and then at –20°C in the lab. Pollinators were mounted on pins, avoiding contact with any surface except the pins themselves. Pollinators were held at the base of their wings and pinned directly on a reversed pin with the help of a clamp, which was cleaned after each use with 70% EtOH. Because our dataset contained mainly Diptera and Hymenoptera (40% and 44% of all pollinators sampled, respectively), we focused on these two orders and exclusively on bees for Hymenoptera. Pollinators were identified by taxonomists to the species level. Hence, our pollinator dataset was based on 658 individuals from 127 species (87 flies and 40

bees; Table S1), with 1–36 individuals per species (median = 2, mean = 5.2, standard deviation (SD) = 7.1), which had interacted with 36 plant species (median interactions per plant species = 5.5, mean = 18.2, SD = 27.7).

#### *Pollinator facial hairiness and area*

Once pollinators had dried, we took images of each one's face using a digital camera (5D Mark IV, Canon, Japan) with a macro lens (MP-E 65 mm f/2.8 1–5× Macro Photo, Canon, Japan). The f-stop was 7.1, exposure time was 1/200 s, and ISO was 160. Images were taken in a white polystyrene box. As a light source, we used two flash units (Speedlite 430EX III-RT, Canon, Japan) at 1/4 of their power connected to a transmitter (ST-E2 Speedlite, Canon, Japan). Mounted on an automated motorized rail (Stack-Shot Macro Rail Package, Cognisys, Traverse City, Michigan), the camera took multiple images of each pollinator's face as it moved toward the insect, with the same distance between each pair of images. This distance ranged from 75 to 350 µm depending on the magnification (3–5×, depending on facial area). Images of each pollinator were stacked using Helicon Focus 7.5.6 software (Helicon Soft, Kharkiv, Ukraine). We calculated the hairiness of pollinator faces using the method and MATLAB script (in MATLAB software v.8.5.0.197613 R2015a) developed by Stavert *et al.* (2016), who calculated hairiness as a Shannon entropy index of stacked macro images. The higher the entropy index, the hairier the insect. In our study, however, the cornea of each ommatidium in the compound eyes was often incorrectly considered a hair, which artificially increased the hairiness index. Consequently, we removed compound eyes from images using GIMP 2.8 software before calculating the hairiness index.

We also had to remove some pollinator species with heavily sculptured heads (e.g. *Halictus tumulorum*, *Lasioglossum paucillum*) because we failed to set the camera and configure MATLAB properly to distinguish cuticle texture from hair. Finally, we removed bumblebees with black hair (e.g. females of *Bombus lapidarius*, *B. terrestris*) because our settings did not allow the MATLAB script to distinguish hairs, probably due to a lack of colour contrast with the black cuticle, a problem also experienced in human facial recognition (e.g. Laytner *et al.*, 2014).

According to Moretti *et al.* (2017), body size includes body length, width, mass, and volume. In our study, we considered only the facial area of pollinators as a proxy of their body size because we focused on facial pollen load. By doing so, we could relate facial area directly to the number of pollen grains present on it, which would not have been true for body size. To this end, we measured the facial area of pollinators (in pixels) using the MATLAB script of Stavert *et al.* (2016) and divided it by the magnification of the lens used when taking images.

To validate the relationship between facial area and body size, we also measured the dry body mass of pinned pollinators. Of the 658 insects in our dataset belonging to 127 species, only 543 belonging to 109 species could be weighed directly, using an analytical balance with a precision of 0.1 mg (Sartorius SE2, Sartorius, Göttingen, Germany). Before weighing, all pollinators were dried at ambient temperature for at least 1 month. We weighed each individual with its pin and then

subtracted the mean mass of the corresponding pin type, which had been estimated by weighing 30 pins per type. For our 10 pin types, the SD of mass ranged from 0.05 to 0.74 mg, and the mean SD of all pin types was 0.36 mg.

#### *Facial pollen load*

Once pollinator images were taken, we collected pollen grains from pollinator faces using small fuchsin jelly blocks produced according to Kearns and Inouye (1993). We collected pollen grains under a stereomicroscope (Motic SMZ-168, Motic, Xiamen, China) at 50× magnification, gently swabbing pollinator faces with the blocks until no more grains were present. Then, the blocks were placed on microscope slides and gently melted at 60°C. Next, they were cooled at ambient temperature with a small piece of metal on it to create thin two-dimensional microscope slides. The small piece of metal was large enough to cover the part of the slide with fuchsin jelly. Then, the piece of metal was removed, and pollen grains were counted at 50× magnification using an optic microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany). For slides with too many grains to count manually, we took images of slides at 50× magnification using the optic microscope connected to a camera (Leica EC3, Leica Microsystems GmbH). We then used the 'cell counter' plugin of Fiji software (v.1.51j) to count the number of grains in each image. We did not identify pollen taxa, so our measure of pollen load did not distinguish mono- versus hetero-specific pollen grains.

#### *Pollen production per floral unit*

The number of pollen grains produced per flower for a subset of the species observed in the field (29 of the 36 species) was retrieved from a dataset of species surveyed in the United Kingdom. The seven lacking species were not taken into account in our study. For the 29 remaining species, the method for extracting and counting pollen was the same as that of Dicks *et al.* (2015). Newly dehiscent stamens were collected (1–460 stamens per tube depending on the species) from flower buds collected in the field and opened in the lab after 24–72 h. They were stored in 1.5-mL Eppendorf tubes filled with 70% EtOH. Samples were vortexed for 30 s and sonicated for 10 min to release the pollen from the anthers, and the plant debris was manually removed after four successive rinses with 200–400 µl of 70% EtOH. The resultant filtrate was centrifuged for 10 min at RCF 14 to produce a concentrated pellet of pollen that was dried in the oven at 60°C for 30–90 min. The pollen pellet was then resuspended in a known volume of 70% EtOH (60–1000 µl, depending on the species). The number of pollen grains from one to two distinct subsamples was quantified by two independent visual counts under light microscopy using a counting chamber composed of 144 squares of 0.0125 µl (modified Fuchs-Rosenthal haemocytometer). The number of pollen grains per flower was calculated as the number of pollen grains per stamen times the mean number of stamens per flower (calculated from five flowers or retrieved from the literature).

The floral unit (FU), an aggregation of flowers that a pollinator can access without flying, could be a more relevant scale at which to study the influence of pollen production on pollinator pollen load. Consequently, on the semi-natural grasslands from which we had sampled pollinators, we counted the number of opened single flowers per FU on 10 individuals per plant species. We calculated pollen production per FU by multiplying pollen production per single flower times the mean number of single flowers per FU.

#### Statistical analysis

We performed all analyses using R software v.3.5.2 (R Core Team, 2019) and generated all graphs using the R package ggplot2 (Wickham, 2009).

*Relationship between pollinator facial area and dry body mass.* We modelled pollinator facial area as a function of dry body mass using a linear mixed model (LMM) with the R package lme4 (Bates *et al.*, 2015). We modelled this relationship with a second-degree polynomial regression (eqn 1). We chose a quadratic function due to non-linearity and the shape of the relationship. We calculated orthogonal polynomial terms with the 'poly' function in R to avoid correlation between them.

$$Y_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + b_i + \varepsilon_i$$

$$\text{and } b_i \sim N(0, \sigma_b^2); \varepsilon_i \sim N(0, \sigma^2) \quad (1)$$

with  $Y_i$  the facial area,  $i$  the pollinator species (1–109),  $\alpha$  the y-intercept,  $x_1$  the pollinator dry body mass,  $x_2$  the square of pollinator dry body mass,  $\beta_1$  and  $\beta_2$  their associated coefficients,  $b_i$  the random term for pollinator species, and  $\varepsilon_i$  residuals.

*Differences in facial area, hairiness, and pollen load between flies and bees.* We described differences in facial area and hairiness between flies and bees using an LMM (eqn 2):

$$Y_{ij} = \mu + \alpha_{ij} + b_i + \tilde{b}_j + \varepsilon_{ij}$$

$$\text{and } b_i \sim N(0, \sigma_b^2); \tilde{b}_j \sim N(0, \sigma_b^2); \varepsilon_{ij} \sim N(0, \sigma^2) \quad (2)$$

with  $Y_{ij}$  the facial area or hairiness,  $i$  the pollinator species (1–127),  $j$  the plant species (1–36),  $\mu$  the y-intercept,  $\alpha_{ij}$  the effect of pollinator taxon (i.e. flies or bees),  $b_i$  the random term for pollinator species,  $\tilde{b}_j$  the random term for plant species, and  $\varepsilon_{ij}$  residuals. Random terms were mutually independent.

We also compared facial pollen load of flies and bees. We used generalized linear mixed models (GLMMs) with a negative binomial distribution due to overdispersion using the R package lme4 (eqn 3). In the rest of our analyses, overdispersion required use of a negative binomial distribution.

$$Y_{ij} | b_i, \tilde{b}_j \sim NB; (\lambda_{ij}, p)$$

$$\text{with } \log(\lambda_{ij}) = \mu + \alpha_{ij} + b_i + \tilde{b}_j$$

$$\text{and } b_i \sim N(0, \sigma_b^2); \tilde{b}_j \sim N(0, \sigma_b^2) \quad (3)$$

with  $Y_{ij}$  the facial pollen load and the other variables the same as those in eqn 1.

*Effects of pollinator facial hairiness and area on total facial pollen load.* To assess facial pollen load of pollinators as a function of their facial hairiness and area, we used a GLMM (eqn 4):

$$Y_{ij} | b_i, \tilde{b}_j \sim NB(\lambda_{ij}, p)$$

$$\text{with } \log(\lambda_{ij}) = \alpha + \beta_1 x_{1ij} + \beta_2 x_{2ij} + b_i + \tilde{b}_j$$

$$\text{and } b_i \sim N(0, \sigma_b^2); \tilde{b}_j \sim N(0, \sigma_b^2) \quad (4)$$

with  $Y_{ij}$  the facial pollen load of pollinators,  $i$  the pollinator species (1–127),  $j$  the plant species (1–36),  $\alpha$  the y-intercept,  $x_1$  pollinator facial area,  $x_2$  pollinator facial hairiness,  $\beta_1$ ,  $\beta_2$  their respective associated coefficients,  $b_i$  the random term for pollinator species, and  $\tilde{b}_j$  the random term plant species. Random terms were mutually independent. We could not add an interaction term between pollinator facial area and hairiness due to model convergence.

We also studied the same relationships for flies alone and bees alone in two separate models. Model equations followed eqn 3 and considered 30 plant species for 88 fly species and 23 plant species for 39 bee species.

To test the interaction between pollinator facial area and hairiness on facial pollen load, we averaged measurements at the species level. We modelled the same relationships with a generalized linear model (GLM) following a negative binomial distribution, with an interaction term added between the two explanatory variables. To run the GLM, we used the R package MASS (Venables & Ripley, 2002) (eqn 5):

$$Y_i \sim NB(\lambda_i, p)$$

$$\text{with } \log(\lambda_i) = \alpha + \beta_1 x_{1i} * \beta_2 x_{2i} \quad (5)$$

with  $Y_i$  the rounded mean facial pollen load of pollinators,  $i$  the pollinator species (1–127),  $\alpha$  the y-intercept,  $x_1$  mean pollinator facial area,  $x_2$  mean pollinator facial hairiness, and  $\beta_1$  and  $\beta_2$  their respective associated coefficients.

*Relationships with pollen production per floral unit.* Finally, to test whether pollen production per FU was related to pollinator facial hairiness and area, we first used an LMM (eqn 6). Of the 658 insects in our dataset, however, only 460 had data available for the pollen production of their interacting plant.

$$Y_{ij} = \alpha + \beta_1 x_{1ij} + b_i + \tilde{b}_j + \varepsilon_{ij}$$

$$\text{and } b_i \sim N(0, \sigma_b^2); \tilde{b}_j \sim N(0, \sigma_b^2); \varepsilon_{ij} \sim N(0, \sigma^2) \quad (6)$$

with  $Y_{ij}$  the facial hairiness or area of pollinators,  $i$  the pollinator species (1–110),  $j$  the plant species (1–24),  $\alpha$  the y-intercept,  $x_1$  pollen production per FU,  $\beta_1$  its associated coefficients,  $b_i$  the random term for pollinator species,  $\tilde{b}_j$  the random term for



**Table 1.** Results of the main linear mixed models (LMMs) and generalized linear mixed models (GLMMs).

Dependent variables	Predictor	Parameter estimate	Standard error	t or z-value	P	Variance of random term	R <sup>2</sup> m	R <sup>2</sup> c	df
Pollinator facial area (pxl)	Intercept	796300	24990	31.870	<2e-16	59180000000	0.70	0.94	97
	Pollinator dry mass (mg)	9261000	371400	24.930	<2e-16				491
	Pollinator dry mass <sup>2</sup> (mg <sup>2</sup> )	-2373000	186400	-12.730	<2e-16				541
Total facial pollen load	Intercept	2.860	0.114	25.156	<2e-16	0.730	0.18	0.34	653
	Pollinator facial area (pxl)	0.723	0.114	6.324	2.54e-10				
	Pollinator facial hairiness	0.322	0.098	3.289	0.001				
Flies total facial pollen load	Intercept	2.237	0.186	12.051	<2e-16	0.415	0.05	0.23	424
	Pollinator facial area (pxl)	0.433	0.104	4.151	3.31e-05				
	1 pollinator species								
Bees total facial pollen load	Intercept	4.186	0.204	20.528	<2e-16	0.605	0.17	0.32	225
	Pollinator facial area (pxl)	0.811	0.183	4.425	9.66e-06				
	1 pollinator species								

The first model (LMM) has *t*-values, while the other models (GLMM) have *z*-values.

plant species, and  $\epsilon_{ij}$  residuals. Random terms and residuals were mutually independent.

We also averaged mean pollen production per FU per pollinator species and modelled mean facial pollen load of pollinators using a GLM (eqn 7). We could not use GLMM due to model assumption issues.

$$Y_i \sim NB(\lambda_i, p)$$

$$\text{with } \log(\lambda_i) = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} \quad (7)$$

with  $Y_i$  the rounded mean facial pollen load of pollinators;  $i$  the pollinator species (1–110);  $\alpha$  the y-intercept;  $x_1$  mean pollinator facial area;  $x_2$  mean pollinator facial hairiness;  $x_3$  mean pollen production per FU of all plant species in interaction with pollinator species  $i$ ; and  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  their respective associated coefficients.

**Model selection and validation.** We selected variables for the models by applying a correlation coefficient (|r|) of 0.6 as a threshold for collinearity (Dormann *et al.*, 2013). When models failed to converge, we dropped the random term that had caused the failure (Barr *et al.*, 2013). We did the same when a singular model fit reflected a variance of random terms close or equal to zero. Model assumptions were assessed following Zuur and Ieno (2016). We selected models using the Bayesian information criterion (BIC) and consequently ran the mixed models using maximum likelihood for parameter estimation (Bates *et al.*, 2015). When  $\Delta\text{BIC} < 2$  for nested models, we retained the most parsimonious ones (Tables S2A–S2F). We tested parameter significance using a *t*-test based on Kenward-Roger's method with the R package lmerTest (Kuznetsova *et al.*, 2017) for LMMs and using the Wald test with the R package lme4 (Bates *et al.*, 2015) for GLMMs. We calculated  $R^2$  for final models using the R package MuMIn (Bartoń, 2019), which quantifies the variance explained by fixed effects (marginal type,  $R^2m$ ) and fixed plus random effects (conditional type,

$R^2c$ ). For GLMMs and GLMs, we calculated pseudo- $R^2$  with the same R package and with the trigramma method due to their logarithmic link function. All pollinator species did not interact with all plant species, which could have biased parameter estimation towards the pollinator and plant species that were represented most in the study.

## Results

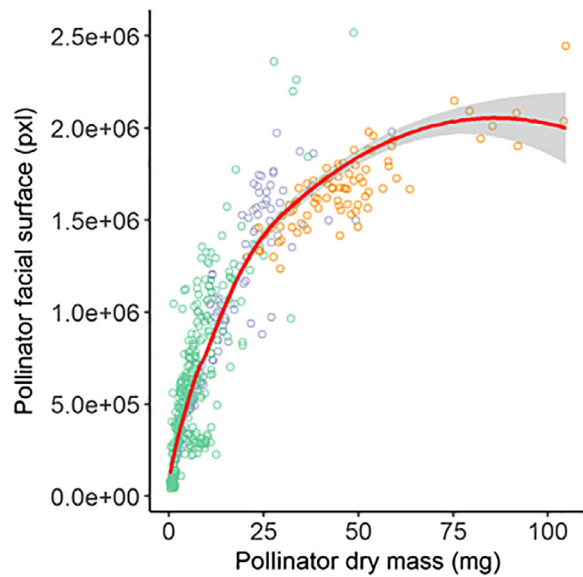
### Relationships between pollinator facial area and dry body mass

For the 543 pollinators from 109 species that we could weigh, we found a significant quadratic relationship between dry body mass and facial area of pollinators (Table 1). The variance explained by both polynomial terms was high ( $R^2m = 0.70$ ). As dry body mass increased, the facial area of pollinators increased until it reached a plateau, which corresponded essentially to bumblebees (Fig. 1).

### Differences in facial area, hairiness, and pollen load between flies and bees

Total facial pollen load ranged from 0 to 1655 pollen grains (mean  $\pm$  SD =  $60.5 \pm 178.0$ ), facial area from 40582 to 2516762 pixels ( $778943 \pm 553691$ ), and facial hairiness from 14.2 to 161.0 ( $72.4 \pm 27.4$ ). Bees had larger faces than flies (*t*-value = 4.9, *df* = 128.1,  $P < 2.58e-06$ ,  $R^2m = 0.16$ ) and showed a bi-modal distribution in our dataset (Fig. 2a). Bees also had hairier faces (*t*-value = 12.1, *df* = 130.8,  $P < 2e-16$ ,  $R^2m = 0.51$ , Fig. 2b) and a larger facial pollen load (*z*-value = 11.9, *df* = 654,  $P < 2e-16$ ,  $R^2m = 0.13$ ; Fig. 2c) than flies.

We found a positive relationship between total facial pollen load and facial area ( $b = 0.723$ ;  $P = 2.54e-10$ ; Fig. 3a) or facial hairiness ( $b = 0.321$ ;  $P = 0.001$ ; Fig. 3b;  $R^2m = 0.18$  for the



**Fig 1.** Pollinator facial area (in pixels) predicted by a linear mixed effects model as a function of pollinator dry mass (mg). Fitted values are projected onto raw data and consider random effects of the model. Grey shading around the regression line shows 95% confidence intervals. Coloured circles are individual flies (green), bees except *Bombus* sp. (violet) and *Bombus* sp. alone (orange). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

model containing both explanatory variables, Table 1). When we added pollinator taxonomic order as an explanatory variable, however, facial hairiness was excluded from the final model, while facial area was retained ( $\Delta\text{BIC} = 6.31$  between the model with all explanatory variables and that without facial hairiness), and the positive relationship between facial pollen load and facial area remained significant ( $b = 0.647$ ,  $P = 4.69\text{e}^{-09}$ ). This last model also had a higher  $R^2m$  (0.24). However, the deviance

explained by the random term for pollinator species (the only random effect in the final model due to convergence) was high for both models ( $R^2c = 0.34$  and  $0.36$ , respectively).

#### *Effects of pollinator facial area and hairiness on total facial pollen load*

When analysing flies and bees separately, we found a positive relationship between total facial pollen load and facial area (respectively,  $b = 0.433$  and  $0.811$ ,  $P = 3.31\text{e}^{-05}$  and  $9.66\text{e}^{-06}$ ;  $R^2m = 0.05$  and  $0.17$ ; Fig. 4, Table 1) but no relationship with facial hairiness (Fig. S1). The significant positive relationships between mean pollen load and mean facial area or mean hairiness were confirmed, with data averaged by pollinator species, but only between mean pollen load and mean facial area when adding taxonomic order as a covariable. However, the interaction between mean facial area and mean hairiness, which was used only in the GLM, was not kept in the final model.

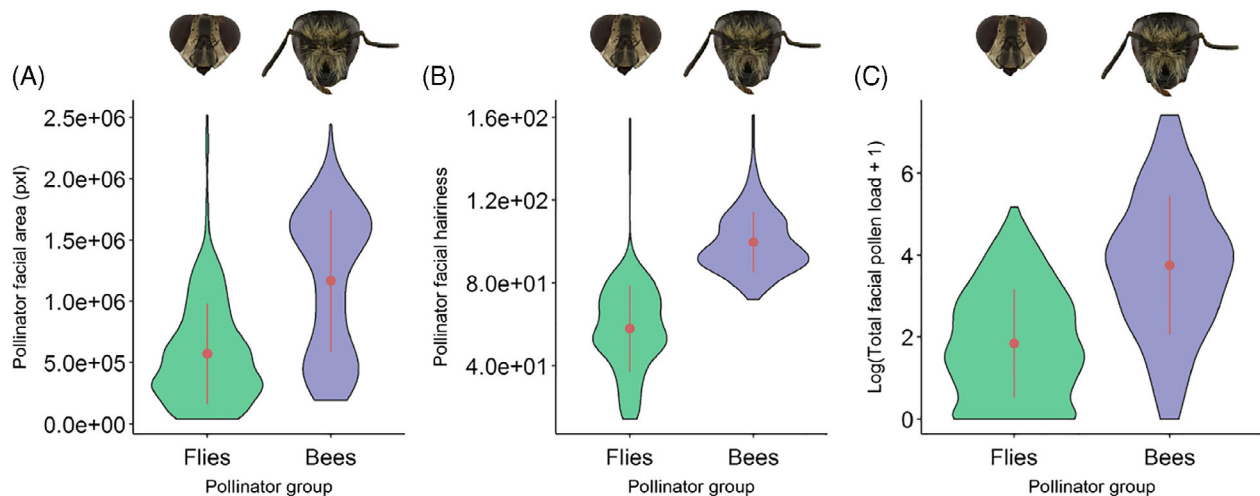
#### *Relationships with pollen production per floral unit*

Pollinator facial area and hairiness were not significantly related to pollen production per FU (respectively,  $b = -9609.2$  and  $-0.345$ ,  $P = 0.324$  and  $0.564$ ; Fig. S2). Likewise, mean facial pollen load was not significantly related to mean pollen production per FU for all plant species visited by each pollinator species ( $b = -0.404$  and  $P = 0.181$ ).

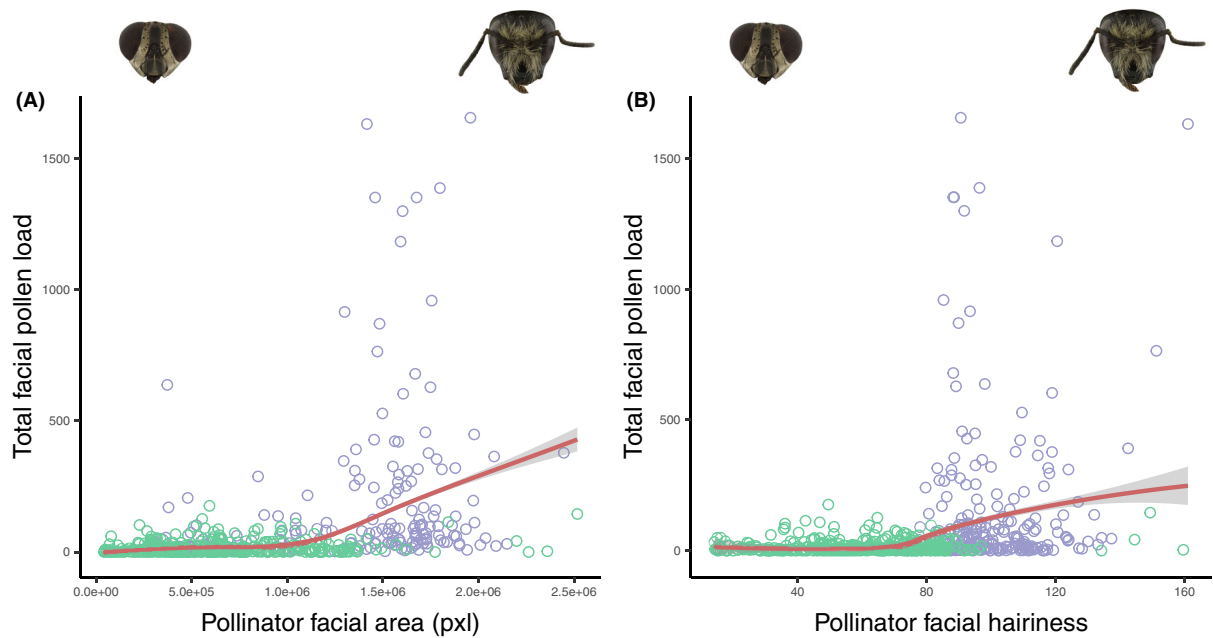
## Discussion

### *Pollinator facial area and dry body mass*

Pollinator facial area and dry body mass were positively linked in our study. Body dry mass is a measurement of



**Fig 2.** Probability density plots (with mean (point) and standard deviation (error bars)) of (a) pollinator facial area (pxl), (b) facial hairiness, and (c)  $\log(\text{total facial pollen load} + 1)$  ( $\log(x + 1)$  scale to increase visibility) of flies (green) and bees (violet). Pollinator faces differ in magnification (5.0 $\times$  for *Sarcophaga carnaria*; 4.5 $\times$  for *Bombus sylvarum*). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



**Fig 3.** Total facial pollen load predicted by a general linear mixed effects model as a function of pollinator (a) facial area (in pixels) (b) and facial hairiness. Fitted values are projected onto raw data and consider other covariables and random effects. See Table 1 for model details. Grey shading around the regression lines shows 95% confidence intervals. Coloured circles are individual flies (green) and bees (violet). Pollinator faces differ in magnification (5.0× for *Sarcophaga carnaria*; 4.5× for *Bombus sylvarum*). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

body size (Moretti *et al.*, 2017). Previous studies found a positive relationship between body size and amount of pollen deposited per interaction with oilseed rape (*Brassica napus*; Phillips *et al.*, 2018) and watermelon (*Citrullus lanatus*; Bartomeus *et al.*, 2018) but not with blueberry *Vaccinium* sp., cranberry *Oxycoccus* sp., or field mustard *Brassica rapa* (Stavert *et al.*, 2016; Bartomeus *et al.*, 2018). We did not use body mass directly in our study but rather pollinator facial area as its proxy, which was a better predictor of pollen load (results not shown). As a result, if pollinator facial area predicts body dry mass well (Fig. 1), facial area could be used as a 'soft trait' (i.e. a trait easy to measure; Lavorel & Garnier, 2002) for pollen load. However, this perspective still needs to be validated.

#### Bees' faces transported more pollen than flies

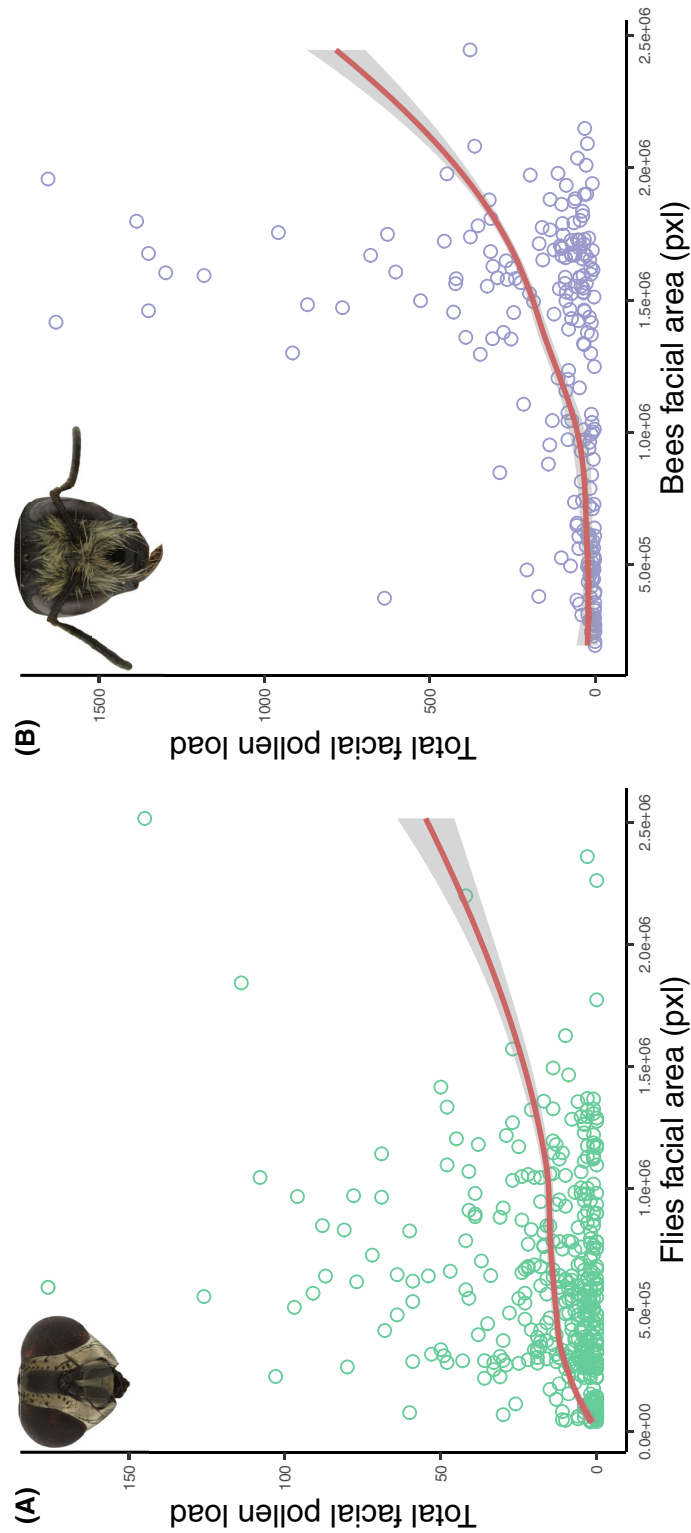
Faces of bees were larger, hairier, and transported more pollen grains than flies. These results could suggest that bees could be more efficient than flies for pollination, similar to previous studies (Kearns & Inouye, 1994; Ballantyne *et al.*, 2017; Willmer *et al.*, 2017). While hairiness could be responsible for this relationship, other collinear phylogenetic pollinator traits may also need to be considered. For instance, flies tend to make less contact between their face and stamens due to their feeding behaviour on most of the flowers. For example, when flies extract nectar or pollen from flowers with short nectar tubes and stamens (e.g. visits to Apiaceae and Asteraceae, which represent, respectively, 52% and 23% of their interactions in our study), they unfold their mouthparts and rarely plunge inside the

flower, unlike most bees (pers. obs.). Consequently, it may be necessary to consider the site of contact between stamens and pollinators more precisely (Harder & Barrett, 1996), especially the latter's legs and ventral parts, to improve the relationship between hairiness and pollen load and to assess pollinator effectiveness (Stavert *et al.*, 2016).

However, flies remain important pollinators, for instance, due to their main role in certain environments (e.g. alpine environments; Lefebvre *et al.*, 2014), or due to their niche complementarity with other pollinators (e.g. thermal niche; Kühnel & Blüthgen, 2015). This pollinator complementarity in face traits and body size in our grassland communities could improve pollination function (architectural complementarity; Blüthgen & Klein, 2011; Garibaldi *et al.*, 2015).

#### Relationships between facial pollen load, area, and hairiness

We found a positive relationship between facial pollen load and facial area, as well as facial pollen load and facial hairiness of pollinators interacting with grassland plant communities, which agrees with the results from Stavert *et al.* (2016) and Phillips *et al.* (2018). To the best of our knowledge, our study is the first to confirm these results for a wide diversity of pollinators and wild plant species and highlights that pollinator hairiness and area are interesting pollination effect traits. The relationship between hairiness and pollen load may be explained mainly by a phylogenetic signal, mirrored in our study by the differences between flies and bees concerning their pollen load and effect trait values. In the context of land use intensification, even though larger pollinators can transport and deposit more pollen



**Fig 4.** Total facial pollen load predicted by a general linear mixed effects model as a function of facial area (in pixels) of individual (a) flies ( $R^2m = 0.05$ ) and (b) bees ( $R^2m = 0.17$ ). Fitted values are projected onto raw data and consider other covariables and random effects. See Table 1 for model details. Grey shading around regression lines shows 95% confidence intervals. Coloured circles are individual pollinators. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



grains per interaction (as our results showed) and thus have a greater pollination function, they could be rarer than smaller pollinators (see Kühnel, 2015), and pollination could be lower due to pollen limitation (e.g. seed production; Bartholomé & Lavorel, 2019). Consequently, future studies should incorporate both differences in pollen limitation among plant species besides pollinator body size to examine pollination outcomes.

Larger and hairier pollinators generally carried more pollen grains on their face than smaller ones, but they also had higher variability in pollen load (Fig. 3). Thus, factors influencing pollen load, such as behaviour, could be more important for estimating pollen load on large hairy pollinators than on small hairless pollinators. Notably, the pollen load of large hairy pollinators may be influenced more by grooming (which removes pollen grains from the body) and its frequency, which is known to influence pollen carryover and to be more common for species harvesting pollen to feed larvae (i.e. female bees in our study; Devaux *et al.*, 2014).

Our models of pollen load based on facial area or hairiness seemed less accurate than those created by Stavert *et al.* (2016), who used the same method to model pollen load of *B. rapa* as a function of facial hairiness. One difference between these studies was our larger dataset (127 pollinator species and 36 plant species vs. 10 pollinator species and 1 plant species for Stavert *et al.*, 2016). Besides the ecological differences in flower anatomy mentioned, methodological differences could also explain this difference in accuracy. We had to correct facial hairiness by removing the eyes of pollinators from images because MATLAB often considered them hairs. This resulted in a more pronounced decrease in the hairiness index of pollinators with larger eyes. Indeed, removed eyes had a null entropy value, while cuticle or hairs always increased the entropy index. We think that keeping eyes in images when measuring hairiness could nonetheless bias measurements; for instance, Stavert *et al.* (2016) calculated a hairiness index for *Eristalis tenax* similar to that of *Helophilus hochstetteri*, perhaps due to extremely hairy compound eyes for the former and an artefact for the latter. The newly protocol proposed by Roquer-Beni *et al.* (2020) to measure hairiness could overcome these issues and even include hair shape, which may be, for instance, plumose on bees (without reflecting an adaptation to pollen collection; Michener, 2007) and may influence the exchange area with stamens.

#### Face pollen load as a good predictor of pollinator pollen load?

We did not count pollen load on the entire body of the pollinator, which could have increased the proportion of low pollen loads in our study, thus flattening the relationship between hairiness and pollen load when hairiness was low. Pollen sampling, counting, and hairiness measurement are time consuming, and we considered as many pollinator species as possible. Some results are in agreement with our methodological choice. For example, Phillips *et al.* (2018) found that, for the 14 pollinator families caught in interaction with *Brassica napus*, pollinator head is a main body part for pollen transport. For the three bee families in their study (Andrenidae, Apidae, Halictidae), the

head is the part of the body that carries the greatest amount of pollen. Stavert *et al.* (2016) found that face hairiness was the best predictor of total pollen load for 10 different pollinator species (bees or flies) in interactions with *B. rapa*. However, it does not mean that face pollen load is also a good predictor of total pollen load. For instance, we used the dataset from Koch *et al.* (2017), which is based on 12 *Apis mellifera* and 12 *Bombus terrestris*. We found that facial pollen load had the lowest correlation with total pollen load in comparison with other pollen loads of other safe sites ( $r = 0.27$  for face; mean of  $r = 0.78$  for other body parts). In their study, total pollen load is best explained by dorsal waist pollen load, although pollen grains can accumulate on the face as much as on other 'safe sites' such as ventral abdomen for *B. terrestris* and ventral and dorsal abdomen for *A. mellifera*. Consequently, the importance of pollinator head or face for pollen transport seems variable.

#### Relationships with pollen production per floral unit and pollinator traits

Contrary to our expectations, the production of pollen per floral unit was not related to the facial pollen load nor the area or hairiness of pollinator species visiting these flowers. Similarly, we did not find any relationship when calculating the same models with pollen production per single flower instead (results not shown). Because the number of pollen grains produced per flower was proposed as an adaptation to the exchange area of the most abundant pollinator species visiting a given plant species (Cruden, 2000), we expected that pollinators visiting highly pollen-productive plants would transport more pollen grains. While a mismatch between the pollinator body part we studied and flower morphology could again be likely here, this lack of relationship could also be explained by the inability to identify pollen grains at the species level.

#### Conclusion

We highlighted facial area and hairiness as two pollinator effect traits that are related to pollen transport, a component of pollination function, for a wide diversity of wild pollinators interacting with grassland plant communities. In order to replace counts of pollen load with measurements of effect traits, we still need to make models more accurate. This goal could be achieved by integrating other effect traits, such as different components of hairiness, but also by taking into account more accurate pollinator phylogeny. Furthermore, we also need to validate the relationship with alternative body parts likely to carry equally or more pollen grains than the face. Future studies should go beyond the relationships between these two effect traits and pollination function by including, for instance, pollination outcomes.

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### Contributions of authors

JG, AM, and SP conceived the idea. JG designed the study advised by AM. JG led the writing of the manuscript. JG adapted technological methods for measuring pollinator traits. JG, CR, AM, and MB collected the data. JG analysed the data. JG, AM, CR, MB, and GM interpreted the results. All authors contributed critically to the drafts and gave final approval for publication.

### Data availability statement

The data that support the findings of this study are available from the authors upon reasonable request.

### Supporting Information

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

**Table S1** List of pollinator species and their frequency in our dataset; n.i.: not identified.

**Table S2** Tables of the selection process for the general linear mixed model or linear mixed models with Bayesian information criterion (BIC)

**Table S2A** Selection process for the general linear mixed model or linear mixed models with Bayesian information criterion (BIC) with pollinator total facial pollen load or pollinator facial hairiness or pollinator facial area as response variable and pollinator taxa (i.e. bee or fly) as explanatory variable.

**Table S2B** Selection process for the general linear mixed model or linear mixed models with Bayesian information criterion (BIC) with pollinator total facial area as response variable and pollinator dry body mass and pollinator dry body mass<sup>2</sup> as explanatory variable.

**Table S2C** Selection process for the general linear mixed model with Bayesian information criterion (BIC) with total facial pollen load as response variable and pollinator facial area and pollinator facial hairiness as explanatory variables.

**Table S2D** Selection process for the general linear mixed model with Bayesian information criterion (BIC) with total facial pollen load of bees as response variable and bee facial area and bee facial hairiness as explanatory variables.

**Table S2E** Selection process for the general linear mixed model with Bayesian information criterion (BIC) with total facial pollen load of flies as response variable and fly facial area and fly facial hairiness as explanatory variables.

**Table S2F** Selection process for the general linear mixed model with Bayesian information criterion (BIC) with total facial pollen load of bees as response variable and bee facial area and bee facial hairiness as explanatory variables.

**Fig. S1** Total facial pollen load as a function of (a) flies facial hairiness and (b) bees facial hairiness.

**Fig. S2** (a) Pollinator facial hairiness and (b) pollinator facial area (pxl) as a function of pollen production per floral unit of the plant species with which they were interacting at the moment they were caught.

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