Supplementary material for: Bees need larger brains to thrive in urban environments

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Supplementary text S1: Brain and body size measurements

Brain size was measured as the weight of the fixed brain for each specimen. First, all collected individuals were kept in cold and sent to the laboratory where they were identified by expert taxonomists and anaesthetised in cold. Then, heads were removed with a scalpel and placed in a fixative solution (4% paraformaldehyde in 0.1 M phosphate-buffered saline -PBS- pH = 7.4) to avoid degradation. Subsequently, brains were extracted from the head capsule and placed on a petri dish after removing the retina from the optic lobes and cleaning the entire brain of all tracheae and fat bodies. By doing this, we ensured that our brain measurements mainly accounted for neural tissue. We then placed the brains on a small piece of Parafilm® and dried the exceeding fixative solution with Kimwipes® tissues. Then, within 4 seconds of the liquid removal, we weighted the brains with the help of a microbalance Sartorius Cubis®. Finally, we calculated the average weight for the species with more than one specimen. Because weighing bee brains is a highly error-prone task, we excluded values that were under or above 1.5 times the interquartile range for each species.

We considered as a proxy of body size the average intertegular span (ITS) per species. ITS represents the distance between the base of the wings (tegulae) on the bee thorax and has been shown to accurately predict body size as it is highly correlated with dry body weight (Cane et al., 2006; Kendall et al., 2019). All ITS measurements were conducted with a stereomicroscope (magnification 16x or 80x) with a calibrated ocular micrometre (resolution down to 0.02 mm).

References:

Cane, J. H., Minckley, R. L., Kervin, L. J., Roulston, T. A. H., & Williams, N. M. (2006). Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. *Ecological applications*, *16*(2), 632-644.

Kendall, L. K., Rader, R., Gagic, V., Cariveau, D. P., Albrecht, M., Baldock, K. C., … & Bartomeus, I. (2019). Pollinator size and its consequences: Robust estimates of body size in pollinating insects. *Ecology and Evolution*, *9*(4), 1702-1714.

Supplementary text S2: Intra- and inter-specific brain body size variation

In average we had 3.31 individuals measured per species with information for both brain and body size. These different individuals within a species tended to have similar values of brain and body size (i.e., low variance) in comparison with the variability found across species. To show this pattern, we calculated the Intraclass Correlation Coefficient (ICC) that estimates how similar or different are the values within the different groups (i.e., species). The ICC values range from 0 to 1. For our case, values close to 0 indicate that the different individual measurements per species tend to be very different to each other and values close to 1 indicate that the measurements within a species tend to be very similar or identical. In order to compute the ICC, we first fitted an intercept-only linear model with the help of the *lmer* function from the *lme4* library version 1.10-34 (Bates, 2010) where we only included a response variable of interest (i.e., brain or body size) and species as a random effect. This allowed us to calculate the variances of interests in order to compute the ICC. We calculated the ICC with the help of the *icc* function from the library *performance* version 0.10.3 (Lüdecke et al., 2021). This function computes the division between the random effect variance and the total variance (i.e., the sum of the random effect variance and the residual variance). We found that for both brain and body size the individual measurements per species tended to be very similar between each other (ICC = 0.947; ICC = 0.931, respectively) but we found high variability across species (see Figure S4). Overall, this indicates that the mean values of brain and body size have biological significance and provides support for investigating macro-ecological patterns across species.

References:

Bates, D. M. (2010). lme4: Mixed-effects modeling with R.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. Journal of Open Source Software, 6(60).

Table S1. Original downloaded cover classes for United States with the respective assigned cover classes and number of bee occurrences found on each of them.

Original NLCD Cover class	Occurrences	Assigned cover class
Hay/Pasture	3837	Agricultural
Developed, Open Space	3470	Urban
Developed, Low Intensity	2844	Urban
Deciduous Forest	2825	Natural
Developed, Medium Intensity	2320	Urban
Cultivated Crops	1038	Agricultural
Mixed Forest	1017	Natural
Woody Wetlands	989	Natural
Developed, High Intensity	962	Urban
Shrub/Scrub	492	Natural
Evergreen Forest	395	Natural
Herbaceous	353	Natural
Open Water	293	Discarded
Emergent Herbaceous Wetlands	278	Natural
Barren Land	143	Discarded

Table S2. Original downloaded cover classes for Europe with the respective assigned cover classes and number of bee occurrences found on each of them.

Original CLC Cover class	Occurrences	Assigned cover class
Discontinuous urban fabric	109779	Urban
Non-irrigated arable land	60722	Agricultural
Pastures	52571	Agricultural
Complex cultivation patterns	20975	Agricultural
Broad-leaved forest	15013	Natural
Land principally occupied by agriculture	10400	Agricultural
Sport and leisure facilities	9534	Urban
Industrial or commercial units	9258	Urban
Mixed forest	8513	Natural
Coniferous forest	7308	Natural
Green urban areas	5924	Urban
Natural grasslands	2900	Agricultural
Moors and heathland	2826	Natural
Water bodies	2578	Discarded
Vineyards	2191	Agricultural
Continuous urban fabric	1875	Urban
Water courses	1848	Discarded
Road and rail networks and associated land	1731	Discarded
Inland marshes	1611	Natural
Mineral extraction sites	1248	Discarded
Fruit trees and berry plantations	1192	Agricultural
Peat bogs	1092	Natural
Construction sites	1085	Urban
Transitional woodland-shrub	1067	Natural
Beaches, dunes, sands	696	Natural
Intertidal flats	576	Natural
Sea and ocean	426	Discarded
Estuaries	422	Discarded
Port areas	376	Urban
Airports	371	Urban
Dump sites	285	Discarded
Salt marshes	166	Natural
Sparsely vegetated areas	33	Natural
Coastal lagoons	23	Natural
Bare rocks	8	Discarded

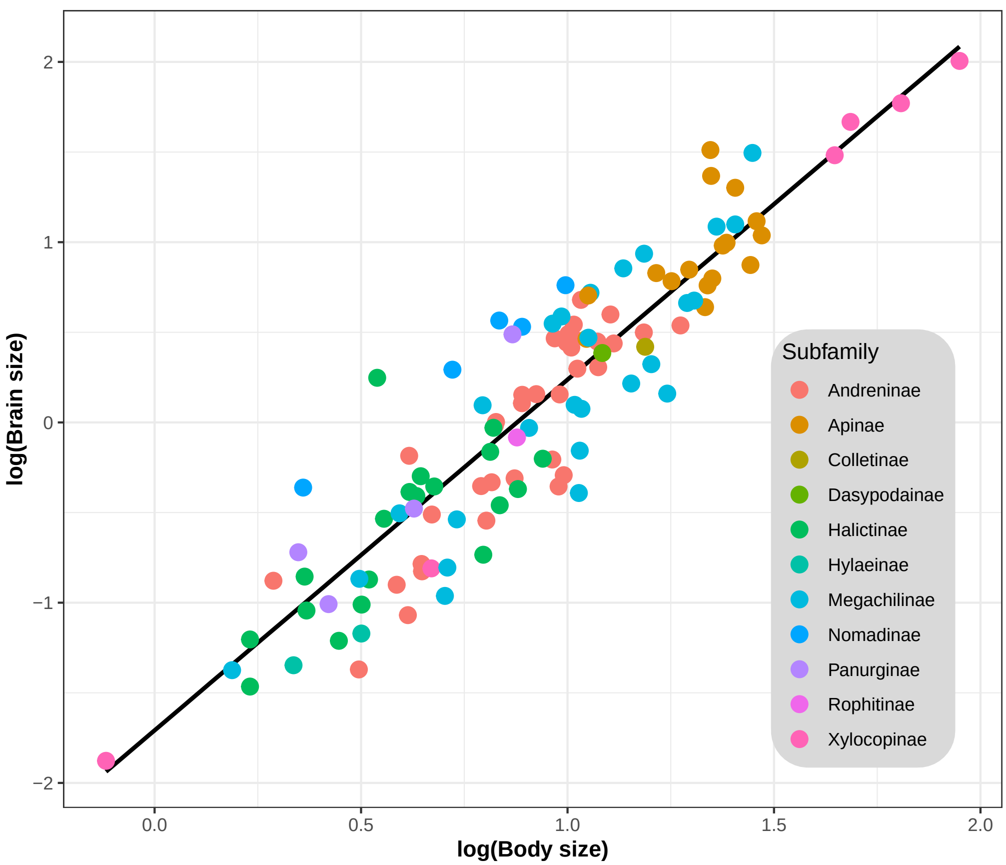

Figure S1. Association between the log-transformed values of brain and body size (N = 116). The different raw values or points are coloured by the subfamily taxonomic rank.

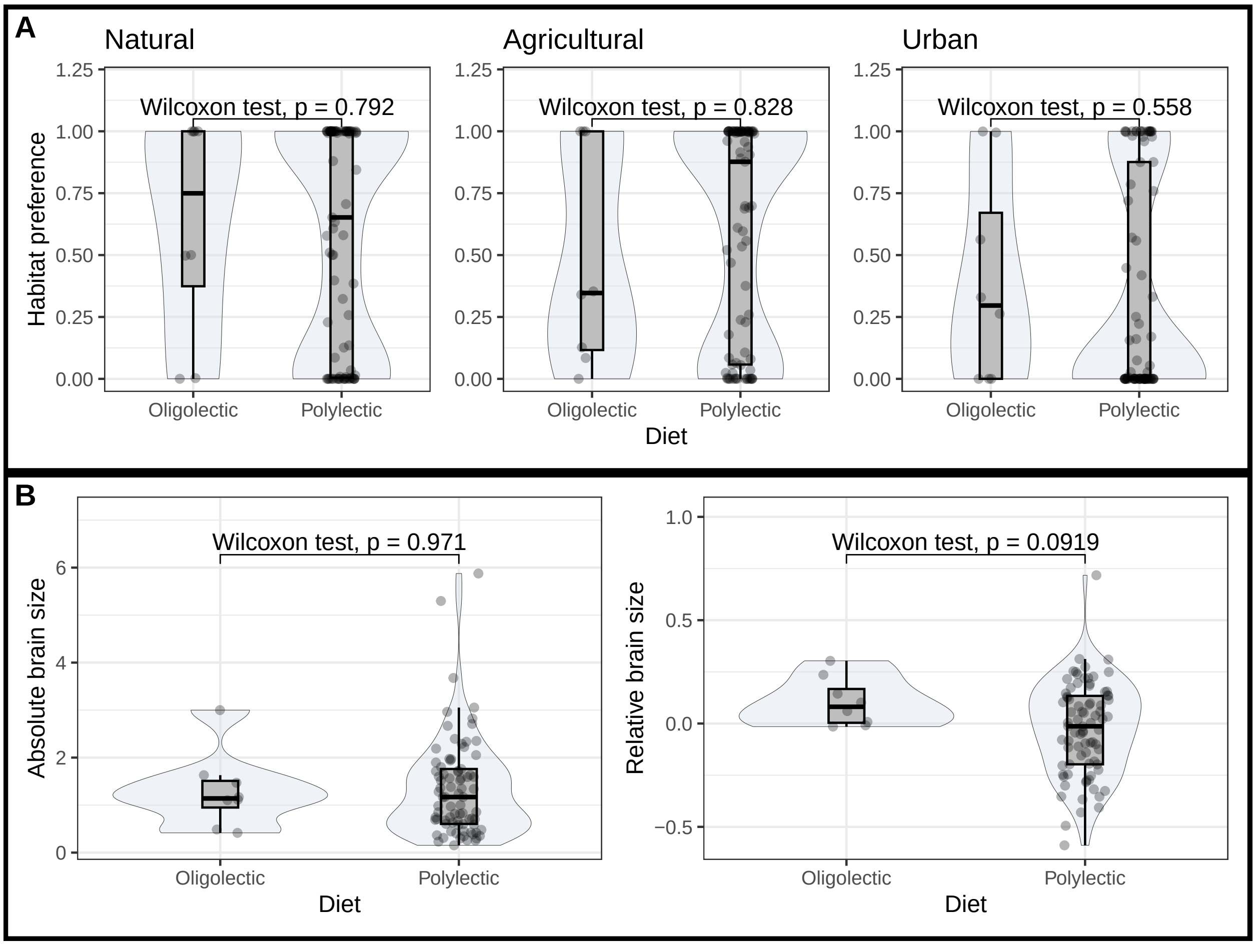

Figure S2. Graphical representation and statistical differences of diet specialisation (i.e., oligolectic and polylectic species) by habitat preference per habitat type (Panel A) and brain size including absolute and relative brain size (Panel B). The graphical representation shows boxplots, raw values and a shaded violin plot to allow a better understanding of the spread, density and number of values by each category. Note that the raw values are jittered horizontally in order to allow a better visualisation of overlapping points. The significance of the statistical differences across values are shown with the resulting P-values of a Wilcoxon test. Interestingly, we see that (i) there are few oligolectic species represented in our dataset; (ii) there are no statistical differences between oligolectic and polylectic species in habitat preference (Panel A) and brain size (Panel B); and, (iii) oligolectic species tend to have larger relative brains and, if anything, show lower preferences by urban habitats, indicating that feeding specialisation is unlikely to be driving the observed association between urban preference and brain size.

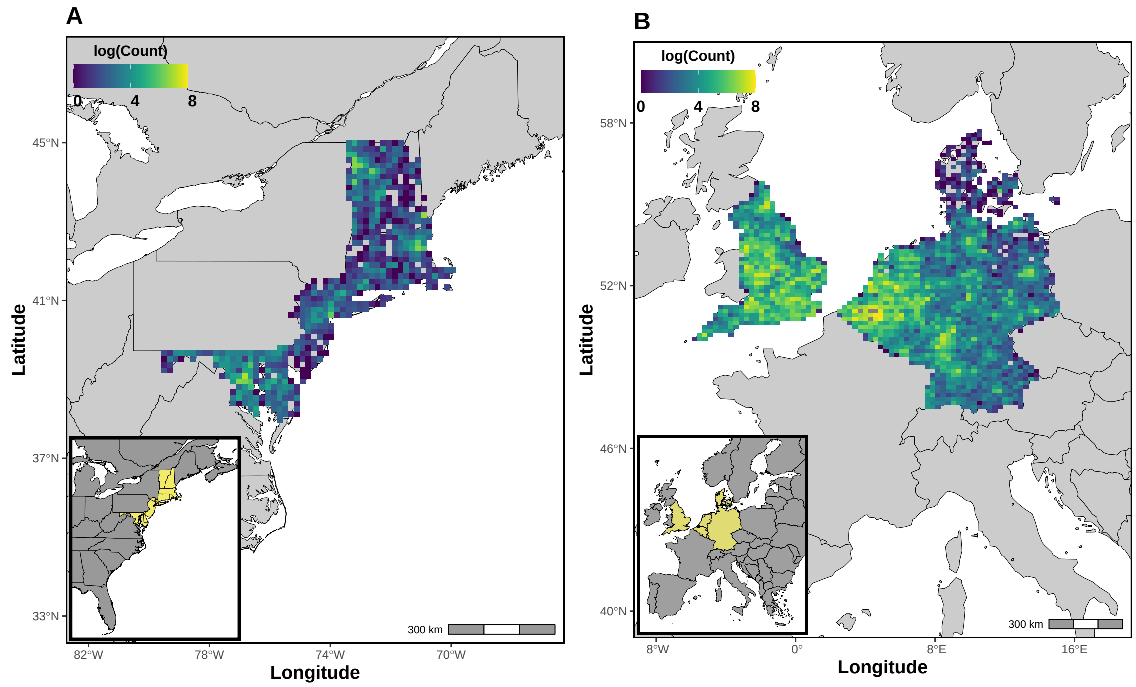

Figure S3. Maps showing the study areas and the number of GBIF occurrences in logarithmic scale for the selected bee species. A) East Coast of United States with records for Vermont, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York metropolitan area, New Jersey, Delaware and Maryland. B) European continent with records downloaded for England, Belgium, Netherlands, Germany and Denmark.

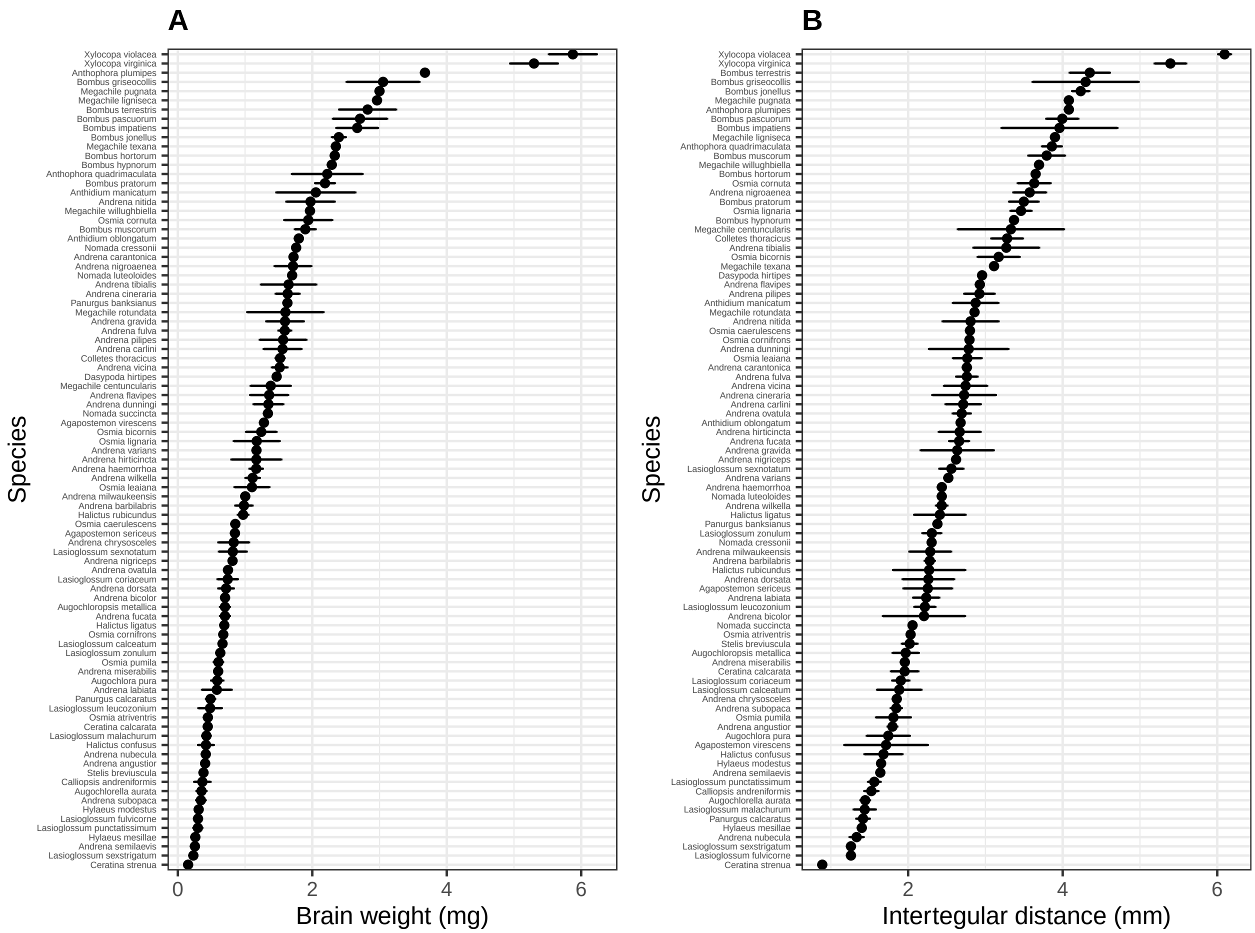

Figure S4. Mean and standard devaition of brain weight and intertegular distance for all the species included in our analyses (N=89).

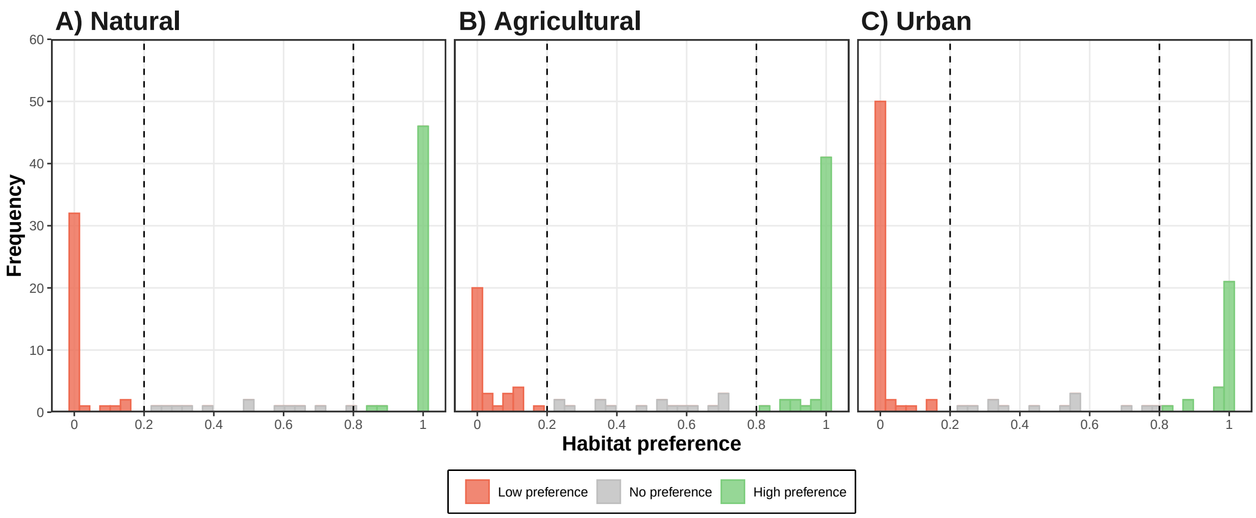

Figure S5. Frequency distribution of the bee preferences for each habitat type (i.e., natural, agricultural and urban). The different habitats are reclassifications from the 2006 land use categories of the Corine Land Cover (CLC) inventory for Europe and the National Land Cover (NLC) database for Unites States.

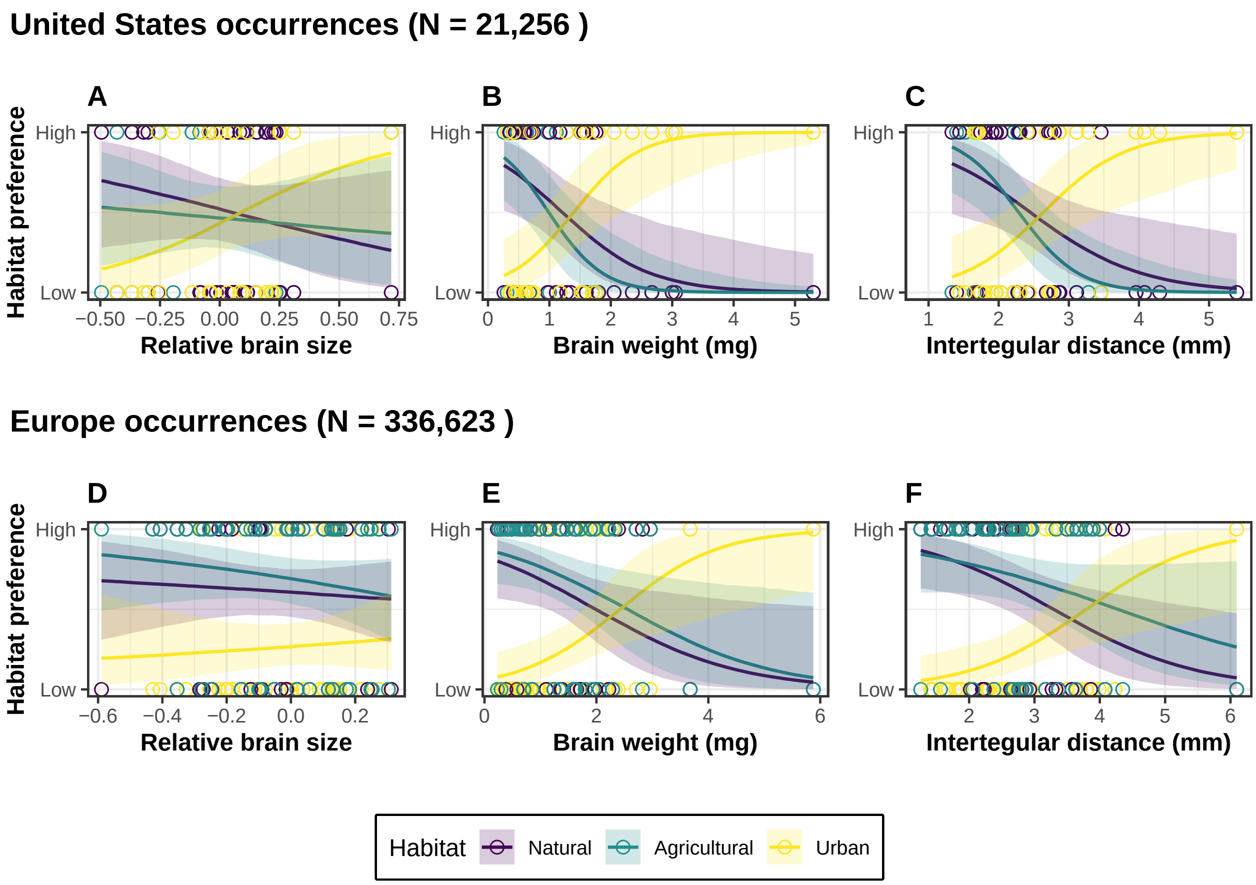

Figure S6. Association between relative brain size (A and D), brain weight (B and E) and intertegular distance (C and F) with habitat preference separated by habitat type (i.e., natural, agricultural and urban) and geographical region (USA in the upper panel and Europe in the lower one). The shaded and coloured areas by habitat type represent 95% credible intervals.