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## Research Article



# When did bumblebees reach South America? Unexpectedly old montane species may be explained by Mexican stopover (Hymenoptera: Apidae)

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A problem for understanding bumblebee biogeography is that if bumblebees dispersed from Asia through North America to South America, if they are poor at long-distance dispersal with establishment over sea, and if the land bridge between North and South America was not established until *c.* 3 Ma BP, then there is an apparent conflict with the divergence among currently endemic South American lineages having been dated as early as 15–17 Ma. Using the first complete phylogenetic trees for all known and accepted extant species of the groups involved, we show how this conflict could be resolved. We suggest that characterizing bumblebees as being associated generally with temperate flower-rich meadows conflates divergent habitat specializations between two early lineages, associated with northern lowland grasslands and with southern montane grasslands respectively, which may have driven divergences in behaviour and in biogeographic processes. First, for most of the lowland grassland group of bumblebees, estimated dates of divergence are consistent with dispersal to South America via the land-bridge corridor that opened at *c.* 3 Ma, followed by extant endemic lineages diverging *in situ* within South America. In contrast, for the second group that occupies montane grassland habitats (and for a few montane lineages of the ‘lowland’ group), we suggest that dispersal to South America at *c.* 3 Ma could be consistent with older divergence for currently endemic species if: (1) many of the extant South American lineages had already diverged outside the region before 3 Ma in neighbouring Mesoamerica; and (2) they had been constrained within the high mountains there, dispersing southwards into South America only once the isthmus corridor had become established; and (3) some of those ancestral montane lineages had become extirpated from Mesoamerica during subsequent warm climatic fluctuations. This interpretation re-emphasizes that biogeographic studies need to consider habitat-specific dispersal models that change through time.

**Key words:** biogeography, bumblebee, dispersal, Panamanian Isthmus, phylogeny, taxonomy

## Introduction

For a century it has been accepted that bumblebees are likely to have originated in Asia and dispersed via North America to South America (Skorikov, 1923). However, there have been few systematic studies of the global biogeography of bumblebees and the details of their history have remained uncertain, especially

concerning when and how they reached South America (Hines, 2008; Kawakita et al., 2004; Santos-Júnior et al., 2022; Williams, 1985, 2015). This uncertainty has been clouded by poorly understood species’ taxonomy and by the consequent difficulties of disentangling species’ distributions (Williams, 1998a). A major problem for understanding bumblebee biogeography is that if bumblebees are poor at establishment after long-distance dispersal (e.g. after crossing broad barriers of ocean or desert, see below) and the land bridge between North

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and South America was not established until *c.* 3 Ma BP (reviewed by O'Dea *et al.*, 2016), then this is in apparent conflict with a recent estimate for the date of divergence among some endemic South American lineages as early as 15 Ma (Hines, 2008) or 17 Ma (Santos-Júnior *et al.*, 2022).

Previous studies of bumblebee biogeography have treated the more than 250 species (Williams, 1998a) as a homogeneous group in terms of habitat associations (Hines, 2008; Kawakita *et al.*, 2004; Santos-Júnior *et al.*, 2022; Skorikov, 1923; Williams, 1985). This may have obscured differences in the biogeographic processes that shaped their history. In this study, we consider whether habitat specializations might differ between the two principal monophyletic groups of bumblebees with consequences for these biogeographic processes and, if so, what differences might follow for our understanding of bumblebee biogeography. To explore these consequences, here we use additional species, gene-sequence data, and updated distribution data to reconsider, in particular, differences in how these two groups may have arrived separately in South America.

### **Bumblebees out of steppe: divergence between lowland and montane grassland bumblebees**

Since Krüger (1917, 1920), bumblebee species have often been interpreted as belonging to one of two large groups (reviewed by Ito, 1985). These two monophyletic groups have been characterized as long-faced and short-faced bumblebees (Cameron *et al.*, 2007: Fig. 1, their LF and SF groups), or as long-tongued and short-tongued bumblebees (Kawakita *et al.*, 2003; 2004: their groups A and B). Tongue length is one of the most important factors affecting bumblebee diet and hence their ecology (Harder, 1983; Heinrich, 1979). However, there is a large overlap between the ranges of absolute (effective) tongue lengths among the workers of the two groups, LF and SF (Williams, 1989, 1991). We propose that there is another (correlated) aspect of bumblebee ecology that is even more directly important for their biogeography.

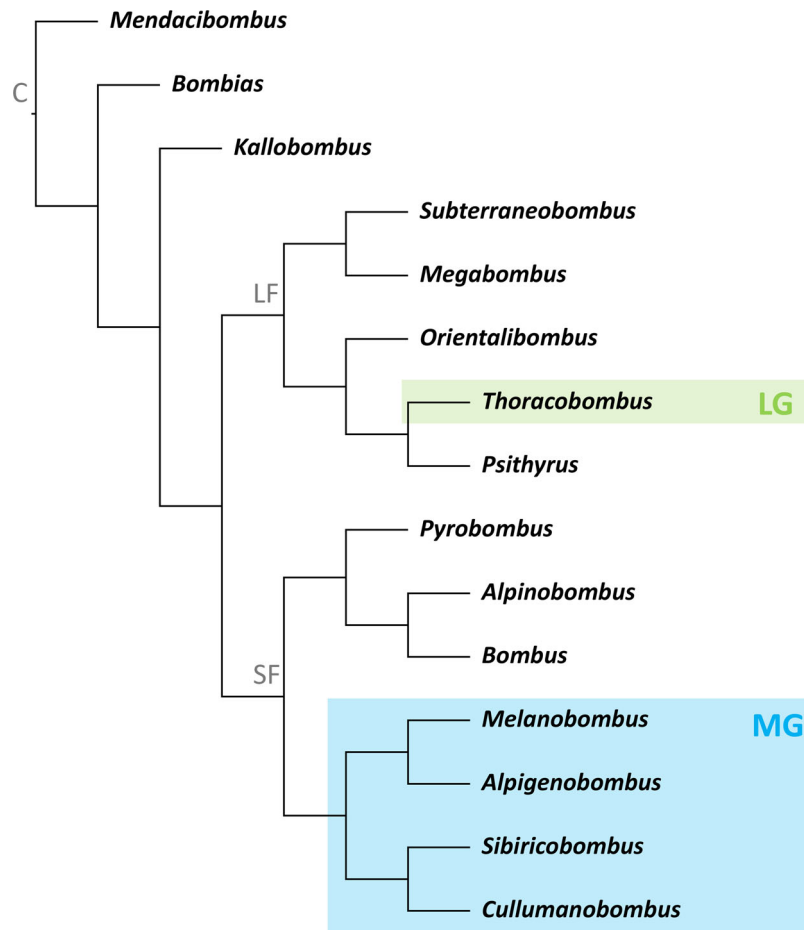
The archetypal habitat for bumblebees has been described as flowery meadow (i.e. flower-rich tall grasslands) (e.g. Heinrich, 1979), especially in cool temperate regions (bumblebees are much less abundant and diverse within closed-canopy forests, deserts, or tropical areas, Williams *et al.*, 2017). However, simplifying the description of bumblebees as grassland insects may be conflating two quite different directions that have been taken in bumblebee evolution: (1) some bumblebees have specialized in lowland grasslands at elevations below forests in some regions, in open steppe or prairie

flatlands; whereas (2) other bumblebees have specialized in montane grasslands at high elevations above the forests, in the subalpine and alpine zones. These two kinds of grasslands occupy two separate zones in both climate space and geographic space. Across a broad range of elevations, closed-canopy forests may be interposed between lowland and montane grasslands, but this distinction may be less clear in more arid regions with few trees, as in eastern Mongolia.

Differences between lowland and montane grassland habitats may have driven divergences in behaviour between different groups of bumblebees. In temperate regions, lowland and montane grassland habitats differ most obviously in the lengths of the annual season that provides the potential for foraging and colony development. In temperate regions, the warm summer foraging season tends to be much shorter at high elevations (although usually with continuous flower availability), reducing the period for colonies to grow and to produce new reproductive queens and males. In the tropics and sub-tropics, seasonal activity patterns at high elevations may depend in part on patterns of food-plant availability, often as a consequence of varying water availability, but also on seasonal patterns of midday heat stress or heavy rain reducing the foraging period (Oyen *et al.*, 2016; Williams, 1991). In contrast, at low elevations in temperate grasslands, the warm foraging season is potentially much longer (also subject to water availability). Lowland grassland bumblebees (e.g. species of the subgenus *Thoracobombus*) have been associated with late-season emergence from hibernation and colony development (Richards, 1975; Sakagami, 1976), a characteristic that has been linked to threatened status (Fitzpatrick *et al.*, 2007; Williams, 2005; Williams *et al.*, 2009). Differences in the length of the available foraging season might in some circumstances also select for different periods of colony development as divergent early-season cycle and late-season cycle reproductive strategies, but this is not well quantified. The different spatial dispositions of lowland and montane grassland habitats are likely to have had different consequences for bumblebee dispersal, an important factor in biogeography.

### **Are bumblebees able to disperse long distances and establish new populations?**

Fundamental to all biogeographic studies is a consideration of the processes of dispersal. For bumblebees, an important distinction needs to be made between short-distance dispersal and long-distance dispersal. This is especially important because the long-term success of dispersal for bumblebees depends on meeting the



**Fig. 1.** Bumblebee subgenera worldwide (Williams et al., 2008) are monophyletic groups of species based on the estimate of the phylogenetic tree from five genes (Cameron et al., 2007) for 208 currently accepted species, although here the estimate is updated and re-drawn from the results of a recent analysis of broad genomic data for 17 currently accepted species representing all subgenera (Sun et al., 2021). Groups of bumblebee subgenera are labelled: C, crown group of all extant bumblebees; LF, the ‘long-faced’ group; SF, the ‘short-faced’ group; LG (green), the large primarily ‘lowland grassland’ group; and MG (blue), the large primarily ‘montane grassland’ group.

genetic requirements for establishing a viable population at the dispersal end point (Williams et al., 2018). Long-distance dispersal events to any distant end point may be rare because of simple geometry if movements are not oriented, but if genetic diversity is also required in order to achieve establishment of a viable new population, then multiple rare events would need to coincide closely in time and space at the distant end point. Such a precise coincidence of rare events at a great distance may be vanishingly unlikely.

For bumblebees, it is the dispersal ability of mated queens (rather than workers or males) that is relevant to establishment of new populations. Bumblebees reproduce in fixed, immobile colonies, which are founded by solitary mated queens after they emerge from hibernation in spring (Heinrich, 1979; Sakagami, 1976). Bumblebee species’ distributions have been known to spread rapidly year after year through suitable habitat

(Crowther et al., 2014; Macdonald, 2001; Macfarlane & Gurr, 1995; R. Schmid-Hempel et al., 2013; Smissen van der & Rasmont, 1999). Furthermore, some species’ populations may show a lack of genetic structure even when distributed very broadly across continents (Cameron et al., 2011; Dellicour et al., 2015; Franoso et al., 2016). Both of these observations point to a high mobility of individuals across large regions of suitable habitat, although this could simply be explained by many steps of short-distance dispersal within suitable habitat. Similarly, observations of spring queens flying in unusually large numbers in the same direction (against the wind) most often along coasts (‘mass migrations’) on the same day and even at coastal sites 200 km apart, or flying towards the sea and flying in from the sea (Fijen, 2021), are not sufficient to demonstrate that queens can fly across oceans, because they could all be explained by individual flights of a few hundred metres,

largely within suitable habitat. However, single queens are occasionally recorded on lightships at up to 30 km offshore (Haeseler, 1974) and on oil platforms at up to 52 km offshore (Prys-Jones *et al.*, 2016). There are records of queens flying up to 50 km from the coast of the Gulf of Finland (Fijen, 2021) and apparently crossing it (i.e. 80 km: Mikkola, 1984). Bumblebees may be strong flyers, but they are heavy, globular and do not glide (unlike dragonflies and butterflies), and their flight is energy expensive – so being blown for hundreds of kilometres on strong winds is unlikely. Furthermore, it is unknown whether queens when they arrive from flights of many tens of kilometres across the sea (for example), assuming that they were able to find food, would be able to regain a suitable physiological state to still be able to found successful colonies.

After long flights, the second obstacle to successful long-distance dispersal is establishment through new colonies that are able to rear more young queens and males. Sex determination in bumblebees depends on heterozygosity (Cook & Crozier, 1995), so mating between siblings from isolated founder colonies would result in the rearing of many diploid males (Duchateau *et al.*, 1994). This effect will be exacerbated because almost all bumblebee species are monandrous (Estoup *et al.*, 1995; R. Schmid-Hempel & Schmid-Hempel, 2000). Males do not forage for the colony and therefore a high proportion of males in early broods would inhibit colony development and represent a severe cost to colony reproductive success. Therefore, successful long-distance dispersal would require several queens in a fit physiological state to arrive at nearly the same place and time in order to achieve the genetic diversity required for establishment.

An apparent example of success from a small number of colonizing queens is the population of *B. terrestris* introduced into Tasmania (Stout & Goulson, 2000). It has been estimated that this population was descended from probably only two mated queens from New Zealand (P. Schmid-Hempel *et al.*, 2007), probably arriving by ship (although there had been two previous recorded interceptions of bumblebees arriving on planes). However, even if this population were descended from just two successful initial colonies, it is widely accepted that many spring queens fail to produce reproductive colonies, so that many more introduced queens may have been required in order to achieve just two successful colonies (the Tasmanian founder queens were also unusually pathogen-free and pre-selected from ‘above-average’ genotypes: P. Schmid-Hempel *et al.*, 2007). The early generations on Tasmania still produced a high frequency of diploid males (Buttermore *et al.*, 1998).

An empirical assessment of the challenge for bumblebee establishment is provided by deliberate attempts to introduce even the ‘invasive’ species *B. terrestris* (Linnaeus) into new sites in New Zealand and Chile. Deliberate introductions (by Macfarlane & Griffin in Macfarlane & Gurr, 1995) showed that as many as 100–150 queens had to be introduced in a season in order to guarantee establishment if sites were not particularly favourable.

All six of the Icelandic bumblebee species are considered to be introductions (from Europe, 950 km to Norway) that arrived by assisted transport with increasing commerce, most of them in recent decades (Prys-Jones *et al.*, 2016). All three of the bumblebee species on the Azores islands (880 km from Madeira, the closest archipelago) are believed to be accidental introductions (Weissmann *et al.*, 2017). *Bombus hypnorum* (Linnaeus) has also become established in Britain (Goulson & Williams, 2001) and in Ireland (O'Donnell, 2018). In this case it has been suggested that queens may have flown unaided the 33 km across the Channel to Britain from the European mainland (Crowther *et al.*, 2014). However, this common garden species might alternatively have arrived while hibernating in flower pots that were transported from garden-plant nurseries in Europe (*B. terrestris* has been found hibernating in the soil in flower pots: McCluskey, 2012). The case of *B. hypnorum* is particularly interesting because this species is very unusual among bumblebees for being polyandrous (Estoup *et al.*, 1995; R. Schmid-Hempel & Schmid-Hempel, 2000). When establishing new small, isolated populations, polyandry should reduce the problem of inbreeding producing early males in place of workers, placing this species at a particular advantage. Assessment of variation within *B. hypnorum* now in Britain implies that genes are still arriving from the continent (Huml *et al.*, 2021). However, continuing arrival of genes might still owe something to assisted transport, with more than 3000 lorries arriving every day in Dover alone, and after the initial population establishment, could also involve the arrival of the smaller males.

Some recent genetic studies have claimed indirect support for long-distance dispersal and establishment for bumblebees, but actually have dealt either with regions that have widespread broadly suitable habitat that requires only short-distance dispersal steps (Cameron *et al.*, 2011; Lepais *et al.*, 2010; Lozier *et al.*, 2011), or with dispersal to islands with pre-existing conspecific populations that should remove the obstacle of rearing males in place of workers (Kraus *et al.*, 2009). In contrast, other genetic studies have concluded that long-distance dispersal over wide barriers is rare for bumblebees (Darvill *et al.*, 2006; Goulson *et al.*, 2011), with *B.*

*muscorum* (Linnaeus) rarely if ever crossing sea barriers of >10 km, although *B. jonellus* (Kirby) appears to readily cross sea barriers of >30 km (Darvill, 2007). However, Goulson (2010) reviewing these results from Britain suggested that species of the subgenus *Pyrobombus* (which includes *B. jonellus*, *B. hypnorum*, but which is not part of our analyses) might be atypically dispersive.

Authors of broader biogeographic studies (who have commented) have all concluded that bumblebees do not generally show long-distance dispersal across broad barriers with establishment, because species often do not occur on more distant islands or in other more remote areas that are considered to have suitable habitat (Estoup et al., 1996; Ito, 1987; Ito & Sakagami, 1980; Lecocq et al., 2017; Panfilov, 1957; Pekkarinen & Teräs, 1993; Skorikov, 1923). That species such as *B. terrestris* can be invasive at all (Orr et al., 2022) demonstrates that their realized distributions are not at equilibrium with their potential global distributions, which is evidence that their long-distance dispersal and establishment has been constrained. On the other hand, this disequilibrium between species' realized and potential distributions does allow biogeographic analyses to reveal patterns in the history of both bumblebee distributions and of changes in the distribution of habitat barriers (Williams et al., 2018).

Consequently, for bumblebees, dispersal and establishment over distances of >80 km can in general be expected only to occur by multiple short-distance dispersal steps to adjacent sites through near-continuous corridors of suitable habitat. High mountains are often uplifted in narrow ranges, as linear chains that constrain the directions for short-distance dispersal within what are, in effect, linear corridors (Williams et al., 2018). In contrast, lowland grasslands may form broad open plains with fewer constraints on the direction of movement for short-distance dispersal. In addition, in periods of rapidly changing climate, mountains may provide more refugia within short distances, for example on opposite sides of the same mountain in areas with different aspect and local climate (Lee et al., 2019; Williams et al., 2016). If establishment after long-distance dispersal is very unlikely, then fine-grained habitat heterogeneity on single mountains could provide better buffering for bumblebee populations against climate change (even if it were more likely to cause population fragmentation and potentially speciation), because it requires only short-distance dispersal for bumblebees to reach refuges. Consequently, lowland and montane grassland habitat might differ not only in how they constrain the direction of dispersal, but also in the extent to which they provide local refuges for resilience to

climate change (Lee et al., 2019). Consequently, we might expect lowland and montane groups to follow differing biogeographic trajectories based on their differing constraints, although it seems likely that both would face challenges in dispersal and establishing over broad barriers of unsuitable habitat.

## Aims

This study examines whether two major monophyletic groups of bumblebees have become associated with different kinds of grassland habitats. It then asks whether this has resulted in different biogeographic histories for the two groups, affecting particularly how they are likely to have dispersed into South America. We estimate the first phylogenetic trees that include all of the currently recognized extant species for the two contrasting groups of species and use these trees to examine geographic patterns in bumblebee evolutionary history.

## Materials and methods

Historical biogeography can help in the understanding of the evolution of organisms through the reconstruction of ancestral distributions (Lomolino et al., 2010). For organisms that do not disperse and establish easily over long distances, ancestral distributions can be estimated by modelling the dispersal process in terms of the more likely corridors for short-distance dispersal (Yu et al., 2010), for example identifying corridors in terms of habitat suitable for bumblebees (Williams et al., 2018). If the two divergent groups of bumblebee species recognized here were associated with two different kinds of habitat, then because there will be different species' distributions in the two kinds of habitat, we should expect different patterns of species richness and therefore different patterns of endemism. We should also expect different dispersal processes because of the different properties of the two kinds of habitat. Therefore, because we need to consider different centres of endemism and different dispersal processes, we employ separate biogeographic analyses for the two groups. Consequently, dated samples of trees are required from phylogenetic analyses for each of the two groups.

## Comparing lowland grassland (LG) and montane grassland (MG) bumblebee groups

For the two large monophyletic groups of bumblebee species that had first been recognized from differences in face and tongue length (LF, SF), these two groups are to some extent distinguished by habitat association (Williams, 2005). The difference is not perfectly

discrete, with the earlier-diverging extant lineages within each of the LF and SF groups appearing less differentiated (more conservative) in their habitat associations, which might confound analyses. Among LF bumblebees, for example, the early-diverging subgenera *Subterraneobombus* and *Megabombus* have only slightly more lowland than montane species (Huang *et al.*, 2015; Williams *et al.*, 2011). Another complication for the LF bumblebees (Fig. 1) is that they include the large subgenus *Psithyrus* (Williams, 1998a: 26 species), species of which are obligate social parasites on both LF and SF bumblebees throughout the northern hemisphere (Williams, 2008), with different behaviour to the social bumblebees (Lhomme & Hines, 2019). Similarly, the SF bumblebees (Fig. 1) include atypical elements such as the subgenus *Alpinobombus*, most species of which specialize in lowland arctic habitats (Potapov *et al.*, 2019; Skorikov, 1937; Williams *et al.*, 2019). In addition, the very large subgenus *Pyrobombus* (66 species, not yet revised at the species level; Hines *et al.*, 2006) and the peculiar subgenus *Bombus s. str.* (23 species; Williams, 2021; Williams, Brown, *et al.*, 2012) occupy especially broad ranges of habitats, from arctic tundra to tropical forest.

To simplify the comparison for this analysis, we compare two large monophyletic groups that include all of the South American representatives by using subgroups that are more clearly differentiated by habitat, which we refer to as the lowland grassland bumblebees (Fig. 1: the ‘LG’ bumblebees, 51 species) and montane grassland bumblebees (Fig. 1: the ‘MG’ bumblebees, 70 species). These LG and MG groups of species (Fig. 1) are: (1) each monophyletic; (2) relatively specialized in their habitats; (3) broadly comparable in numbers of species and age; and (4) still include all of the bumblebee lineages that have reached South America.

### Compiling the species list and distribution data

Work on a catalogue of bumblebee names and a global checklist of known and accepted bumblebee species by PW began in 1980, with lists summarizing progress (Williams, 1985, 1998a) and web pages (<https://www.nhm.ac.uk/bombus>). Several recent revisions of particular subgenera or of faunas worldwide have sought clarifications on species’ status, enabled specimen identification from both morphology and barcodes, as well as providing published barcodes (Brasero *et al.*, 2021; Williams, 2021, 2022; Williams *et al.*, 2012, 2014, 2016, 2019, 2022). *Bombus rubriventris* Lepeletier is considered probably extinct (Williams, 2015). Some taxa of uncertain status and some likely but undescribed species are known from Asia and Mesoamerica, although these cannot be revised

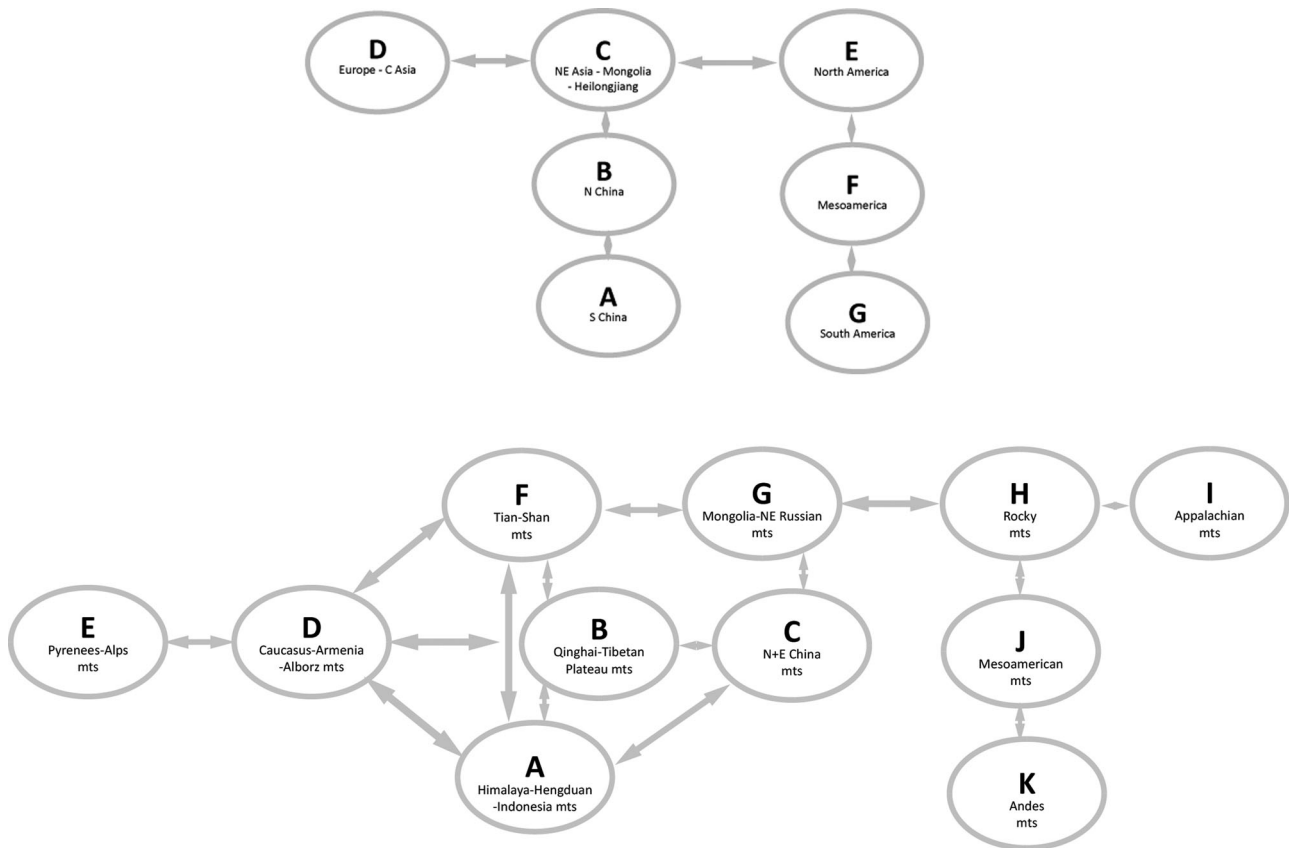
and included here. They are unlikely to affect our main conclusions. In cases where a species’ status and name have been revised since Cameron *et al.* (2007) (PW identified or checked all of the specimens for that analysis), the revised species and name is included here for the phylogenetic analysis.

As an example within the LG group, the Brazilian *B. brevivillus* Franklin and the recently described *B. applanatus* Françoso *et al.* (2016) belong to an assemblage of difficult, entirely black South American bumblebees (including *B. morio* (Swederus), *B. pullatus* Franklin, and some *B. pauloensis* Friese), raising the question as to whether both should be included. The clearest diagnostic morphological characters described for distinguishing *B. applanatus* from *B. brevivillus sensu* Françoso *et al.* (2016) (specimens of the latter could not be examined so its identity is unclear) were that *B. applanatus* females have (1) the clypeus medially nearly flat with the sides sharply angled and (2) the hind tibia posterior fringe is unusually short. Both character states have previously been considered uniquely diagnostic for *B. brevivillus sensu* Franklin (confirmed by PW from examining the type specimens of both). Consequently, no characters are now known that distinguish *B. brevivillus sensu* Franklin and *B. applanatus*. Therefore, because *B. applanatus* appears to be conspecific with *B. brevivillus sensu* Franklin, they are considered here to be synonyms. Sequences provided for the taxon *applanatus* can now be used to represent the species *B. brevivillus sensu* Franklin.

Work on a database of species’ distributions by PW began in 1988, aiming for consistent and reliable species’ identifications. This was based primarily on specimens examined in major collections in Europe, Asia and North America. Data were also added from many publications when records could be checked, including work on the bumblebees of China (Williams *et al.*, 2017), the Himalaya (Williams, 2022), Europe (Rasmont *et al.*, 2021; Rasmont & Flagothier, 1996), North America (Williams *et al.*, 2014) and Mesoamerica (Labougle, 1990). Hines (2008) used an earlier and simplified version of these data from PW’s NHM web pages (based on Williams, 1998a). Records have been summarized using a coarse-scale equal-area grid for maps of species’ richness (maps in Williams, 1998a; Fig. 2) and endemism (measured as range-size rarity, Williams, 1998a).

### Dated species phylogenetic tree estimation

The most widely accepted estimate of the phylogenetic tree for bumblebee subgenera and species has been obtained from five genes (Cameron *et al.*, 2007) that were aligned separately and then concatenated (these



**Figs 2, 3.** Diagrams representing within-habitat dispersal models for (2) lowland corridors for the lowland grassland group and (3) montane corridors for the montane grassland group, encompassing a set of the short-distance dispersal events permitted between the areas of endemism for bumblebee species, based on their geographic proximity and the likely disposition of corridors with suitable habitat and favourable climates for at least part of the history of bumblebees. These models are open, allowing potential for free dispersal in either direction in all time periods.

data are available for re-analysis). The five genes are mitochondrial 16S rRNA ('16S') and nuclear long-wavelength rhodopsin copy 1 ('opsin'), elongation factor-1 alpha F2 copy ('Ef-1 $\alpha$ '), arginine kinase ('ArgK') and phosphoenolpyruvate carboxykinase ('PEPCK'). The resulting tree has now been largely supported and refined for subgenera (Fig. 1) using broadly sampled genomes assembled for a representative of each subgenus (Sun et al., 2021).

The list of world bumblebees as species accepted here includes 289 species (<https://www.nhm.ac.uk/bombus>, updated with comments 2022), only 208 (72%) of which were included in the Cameron et al. (2007) tree. Data for the five genes are still unavailable for some species, although Wang et al. (2020) did provide data for two more species of LG and MG (*B. opulentus* Smith and *B. pyrosoma* Morawitz) and Françoso et al. (2016) provide data for *B. brevivillus* (under the name *B. applanatus*). This extended Cameron et al. (2007) tree can be used as a 'backbone' (Talavera et al., 2022;

Trunz et al., 2016), on which all of the remaining species accepted here can be interpolated by using data for the rapidly evolving cytochrome *c* oxidase subunit I ('COI') gene from the GenBank and BOLD databases (boldsystems.org). The data aligned for each gene separately include a total of 5420 base positions with a maximum of data for 4475 base positions. The COI sequences were obtained using standardized protocols (Hebert et al., 2003). For full data see the online material.

Trees were estimated from the combined six-gene dataset (excluding the morphological data from the Cameron et al., 2007 data) using the Bayesian procedure BEAST (version 2.6.6, Drummond & Bouckaert, 2015) for: (1) all *Bombus* (to obtain point and interval estimates of the crown ages for the subsequent analyses, see below); (2) the lowland grassland group; and (3) the montane grassland group. The best substitution model from the Bayesian information criterion in Mega6 (version 6.06, Tamura et al., 2013) is the general time-



reversible model with a gamma-frequency distribution of changes among sites, with the XML settings scripted using BEAUTi (version 2.6.6, Drummond & Bouckaert, 2015), with the site model with four gamma categories, a log-normal relaxed clock model, a calibrated Yule model, a prior added to identify the outgroup (for *Bombus*, *Plebeia frontalis* (Friese); for LG, *B. (Psithyrus) chinensis* Morawitz; for MG, *B. (Bombus) ignitus* Smith) and monophyletic ingroup (other closely related species were also tested with similar results), with the MCMC set to 100 million generations sampled every 10,000 generations. Analyses were repeated in multiple separate runs to confirm that consistent results were obtained. Tracer (version 1.6.0, Drummond & Rambaut, 2007) was used to examine the trace files. TreeAnnotator (version 2.6.6) was used to find a maximum clade credibility tree with mean node heights after burn-in. Trees were drawn with FigTree (version 1.4.4, <http://tree.bio.ed.ac.uk/software/figtree/>) and Illustrator (Adobe, version 26.0.1).

Age calibration of the tree is based on the dated tree of Hines (2008), which used the Cameron *et al.* (2007) data together with molecular rates and point calibration from fossils from outside of the genus *Bombus*. Fossils within *Bombus* are not very informative. Only the fossil of *B. trophonius* Prokop *et al.*, 2017 contributes a date within the scope of this study, which, at 20 Ma for a position close to the lineage to the MG group *Melanobombus* + *Alpigenobombus* + *Sibiricobombus* + *Cullumanobombus* (Dehon *et al.*, 2019), is consistent with the Hines (2008) estimate.

The dates for the selected LG and MG nodes were obtained by re-estimating the tree for the 208 currently known and accepted species of the genus *Bombus* that are represented in the data of Cameron *et al.* (2007). We follow the procedure of Hines (2008) in fixing the date of the crown divergence within the genus *Bombus* at 34 Ma in order to estimate a crown date here for LG with a mean age of 13.01 Ma (95% highest posterior density interval 6.2 Ma, approximated by sigma 1.54 Ma) and a date for MG of 14.90 Ma (HPD= 7.0 Ma,  $\sigma$ =1.8 Ma). This two-step process makes the extraction of separate estimates of 10,000 sample trees for the LG and MG groups tractable for the two subsequent biogeographic analyses.

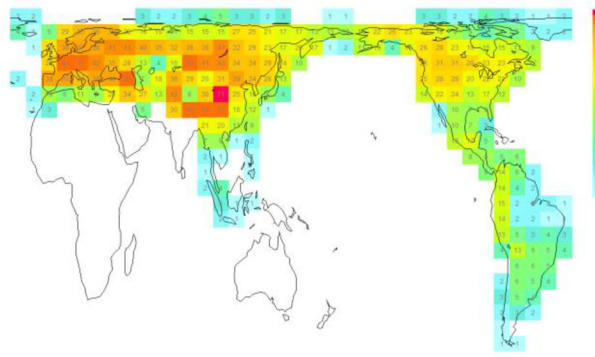
### Ancestral distribution estimation

To seek the most likely explanation for current bumblebee distributions if only short-distance dispersal were possible (Williams *et al.*, 2018), separate models of potential corridors for dispersal (assuming suitable climates and habitats in all appropriate periods) are defined

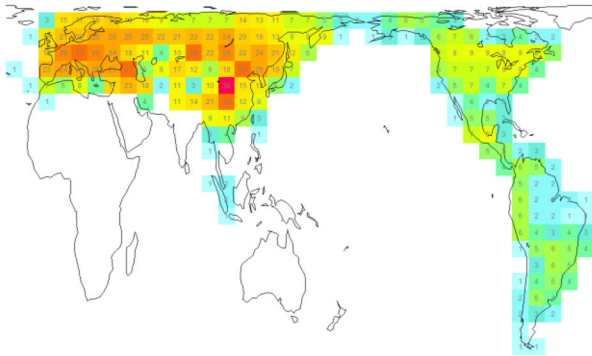
for the LG and MG groups in their different habitats (Figs 2–3). These models summarize a set of short-distance-dispersal events (excluding long-distance dispersal ‘jumps’ over wide barriers) permitted between neighbouring areas of endemism assuming that suitable habitat and climate is present within these corridors.

Area units are chosen by agglomerating species’ distribution data into a few areas with shared, often nested, faunas with unique endemic species or combinations of species. Such areas often appear as areas of high species richness in Figs 7–8 and as areas with high endemism scores in Figs 9–10. The number of areas recognized within a species’ range has to be kept low because the biogeographic analysis does not have mechanisms by which two daughter species can both inherit ranges consisting of many areas (Lamm & Redelings, 2009; Ree & Sanmartin, 2009). Ancestral ranges are constrained to be a maximum of three contiguous areas so as: (1) to exclude long-distance-dispersal jumps between non-contiguous areas; (2) to not exceed the size (as number of area units) coded for current ranges of species; (3) to offset the bias of analyses towards combining all areas from daughter species into widespread ancestral ranges; and (4) to prevent reconstructed ranges from being artificially highly disjunct (Lamm & Redelings, 2009; Ree & Sanmartin, 2009; Ree & Smith, 2008; Ronquist, 1996). This constraint is more restrictive for the LG analysis because species in this group tend to have larger current geographic range sizes than MG species (LG species range size mean 15.8 median 8 equal-area grid cells, MG species range size mean 7.7 median 5 equal-area grid cells; large ranges of LG species may be related to postglacial expansion across very extensive and interconnected lowland habitats: Dellicour *et al.*, 2017; Reinig, 1935, 1939). Therefore, in order to keep range sizes down to three areas of endemism, those areas cannot be as finely resolved within the Old World. Consequently, the spatial resolution of biogeographic analysis for the LG group (with these algorithms) is limited by the much larger distribution ranges of some LG species compared with MG species.

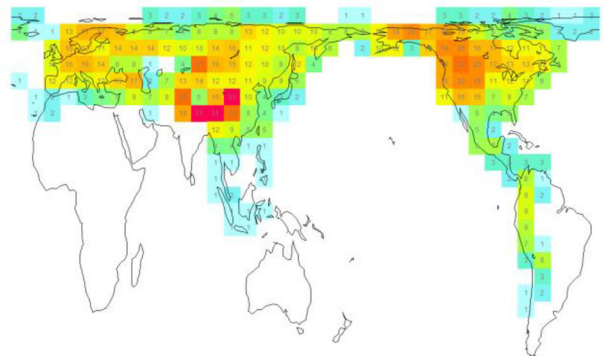
To assess explanations of current distributions from phylogenetic trees, dispersal and extinction, we use the statistical version (from samples of trees) of the BioGeoBEARS methods (Matzke, 2013a, 2013b, 2014) from a library in *R* (*R* Core Team, 2017) within the RASP package (version 4.2, accessed 2020: Yu *et al.*, 2020). S-BioGeoBEARS uses a sample of 10,000 ultrametric trees from BEAST. The DIVALIKE + J option was selected a priori because it assumes that distribution changes occur at nodes (associated with speciation: Yu *et al.*, 2020), as S-DIVA has been found to perform better with bumblebee data (Williams *et al.*, 2017) than



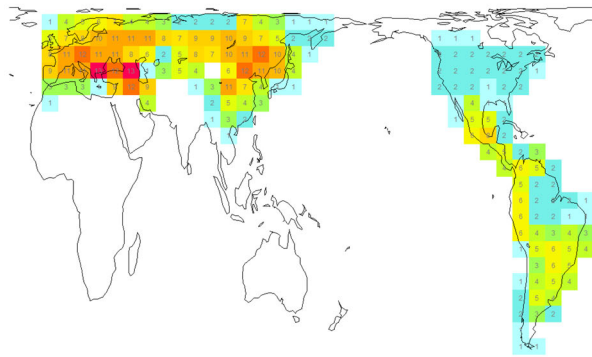
4: C 289 species



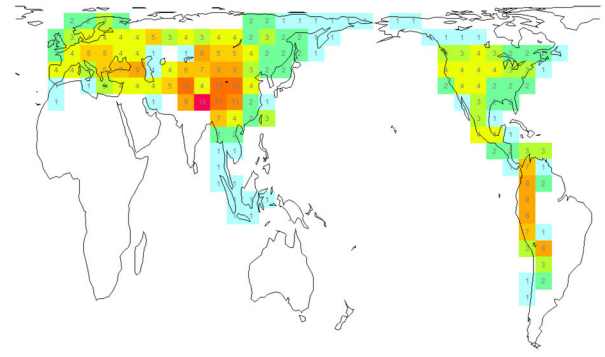
5: LF 115 species



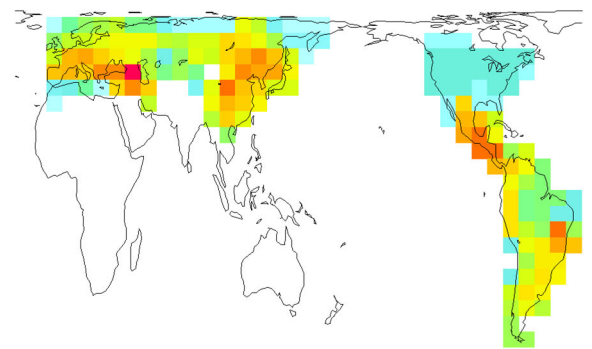
6: SF 158 species



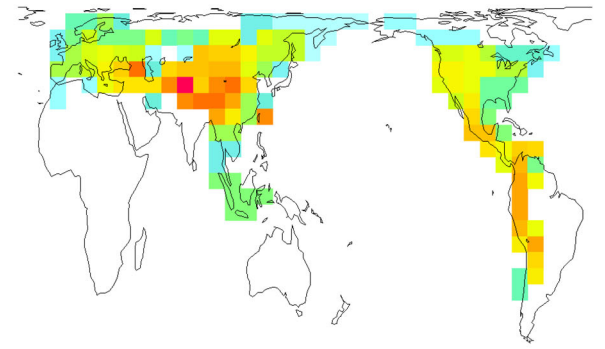
7: LG 51 species



8: MG 70 species



9: LG endemism



10: MG endemism

S-DEC (Yu *et al.*, 2014), which assumes changes occur along branches (S-DIVA has also been applied to the present data although the results did not change the inferences).

The explicit models for dispersal among areas of endemism are shown in Fig. 2 for LG bumblebees and in Fig. 3 for MG bumblebees. There is no order implicit in the area names: the polarity of dispersals is estimated from the trees and the outgroup. The permissible dispersal corridors in these models for LG are: AB ABC BC BCD BCE CD CDE CE CEF EF EFG FG (e.g. ABC means that a distribution including the combination of all three regions A, B, and C is permitted by the model for an ancestral lineage; any combinations that are not included in this list are not permitted); and for MG are: AB ABC ABD ABF AC AD ADE ADF AF AFG BC BCD BCF BCG BD BDE BDF BF BFG CFG CGH DE DEF DF DFG FG FGH GH GHI GHJ HI HIJ HJ HJK JK. Because of the geological and climatic uncertainties, these models are fully open, allowing free dispersal both in either direction and in all time periods.

## Results

### Areas of richness and endemism for LG and MG bumblebees

The greatest species richness of the groups LF and LG is associated (Figs 5, 7) with the more northern steppe areas of Mongolia and north-eastern China, Siberia, and eastern Europe, with a smaller peak also in southern Mexico.

The greatest species richness of the groups SF and MG is associated (Figs 6, 8) with the more southern mountain areas of the high Qinghai-Tibetan Plateau, Central Asia, southern Europe, and the western New World (in North America the high values in Fig. 6 are contributed especially by the subgenus *Pyrobombus*, while in South America the high values in Fig. 8 are contributed by the subgenus *Cullumanobombus*).

The distribution of richness is more similar between LF and SF bumblebees (Figs 5, 6: Spearman rank correlation  $\rho = 0.69$ ) than it is between LG and MG bumblebees (Figs 7, 8: Spearman rank correlation  $\rho = 0.41$ ). Using the LG/MG comparison for our analysis therefore

represents a greater contrast in spatial distributions than LF/SF, which is consistent with reducing the overlap in habitat use between the two groups.

For both LG and MG groups, there is a major centre of endemism in southern Mexico (Figs 9–10).

### Complete dated phylogenetic trees for LG and MG bumblebees

There are slightly more MG (70) than LG (51) bumblebee species (37% difference), with only a small difference (15%) in the age of the most recent common ancestors (MRCAs) of these groups. Estimates of dated trees for all species of the LG and MG groups with revised species' names are shown in Figs 11–12. Within 100 million MCMC generations, the traces had converged on stable solutions long before a 5% burn-in. After the burn-in the effective sample sizes were high, with a minimum value of 2581 among all parameters for the LG tree and 1357 for the MG tree. Some nodes on the trees are poorly supported (support  $< 0.66$  indicated by age estimates in grey in Figs 11–12), although these nodes do not affect the principal biogeographic inferences.

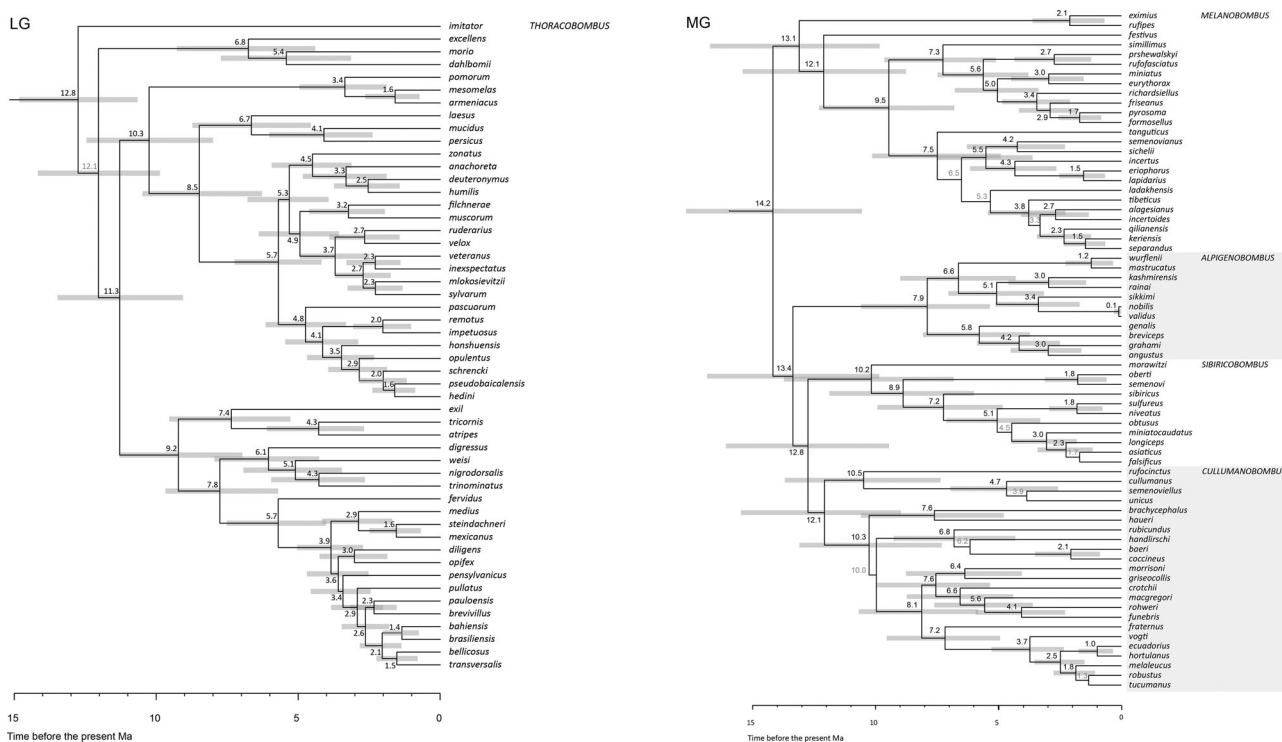
The larger South American subgroups with extant distributions endemic to South America (subgroups in the lower part of each figure) for the LG subgroup *pullatus-transversalis* (posterior support 1.0) had a MRCA at *c.* 2.9 ( $\pm 1.1$ ) Ma. For the MG group, the result is slightly less clear. The most recent subgroup to include all of the currently endemic South American lineages, *rubicundus-tucumanus*, is poorly supported (posterior support 0.49) at *c.* 10.0 Ma, however the only slightly older and more inclusive subgroup *brachycephalus-tucumanus* is very strongly supported (posterior support 1.0) at *c.* 10.3 ( $\pm 2.8$ ) Ma.

### Ancestral distributions for LG and MG bumblebees

Estimates of ancestral distributions for lineages at nodes on the dated trees for the LG and MG groups are shown in Figs 13–14.

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**Figs 4–10.** Bumblebee species richness for: (4) all species; (5) long-faced group; (6) short-faced group; (7) lowland grassland group; (8) montane grassland group; (9) endemism as range-size rarity (Williams, 1998b: sum of the inverse of the species' range sizes) for the lowland grassland group; (10) endemism as range-size rarity for the montane grassland group. Even for a globally well-sampled group such as bumblebees, using a coarse-scale equal-area grid reduces species-area effects, reduces the effects of sampling heterogeneity (species-accumulation curves for these large grid cells are more nearly asymptotic), and smooths the effects of local habitat patchiness. The grid is based on intervals of  $10^\circ$  longitude, which are used to calculate graduated latitudinal intervals in order to provide equal-area cells (each cell has an area of  $\sim 611,000 \text{ km}^2$ ). The colour scale (top right) has equal-frequency richness classes within each map, so colours are not comparable among maps. Cylindrical orthomorphic equal-area projection (excluding Antarctica) with north at the top of the map.



**Figs 11, 12.** Bayesian estimates of phylogenetic trees for all extant currently recognized species of (11) the lowland grassland (LG) group and of (12) the montane grassland (MG) group, analysed with BEAST from six genes (not morphology) over 100 million MCMC generations (5% burn-in) including recently revised species' taxonomy and names. Numbers at nodes are estimates of the age of a node in Ma before present, with grey node bars representing the 95% highest posterior density interval of the age estimates (the age numbers are shown in grey where posterior support for the node is  $<0.66$ ). Outgroups have been removed (LG, *B. chinensis*; MG, *B. ignitus*).

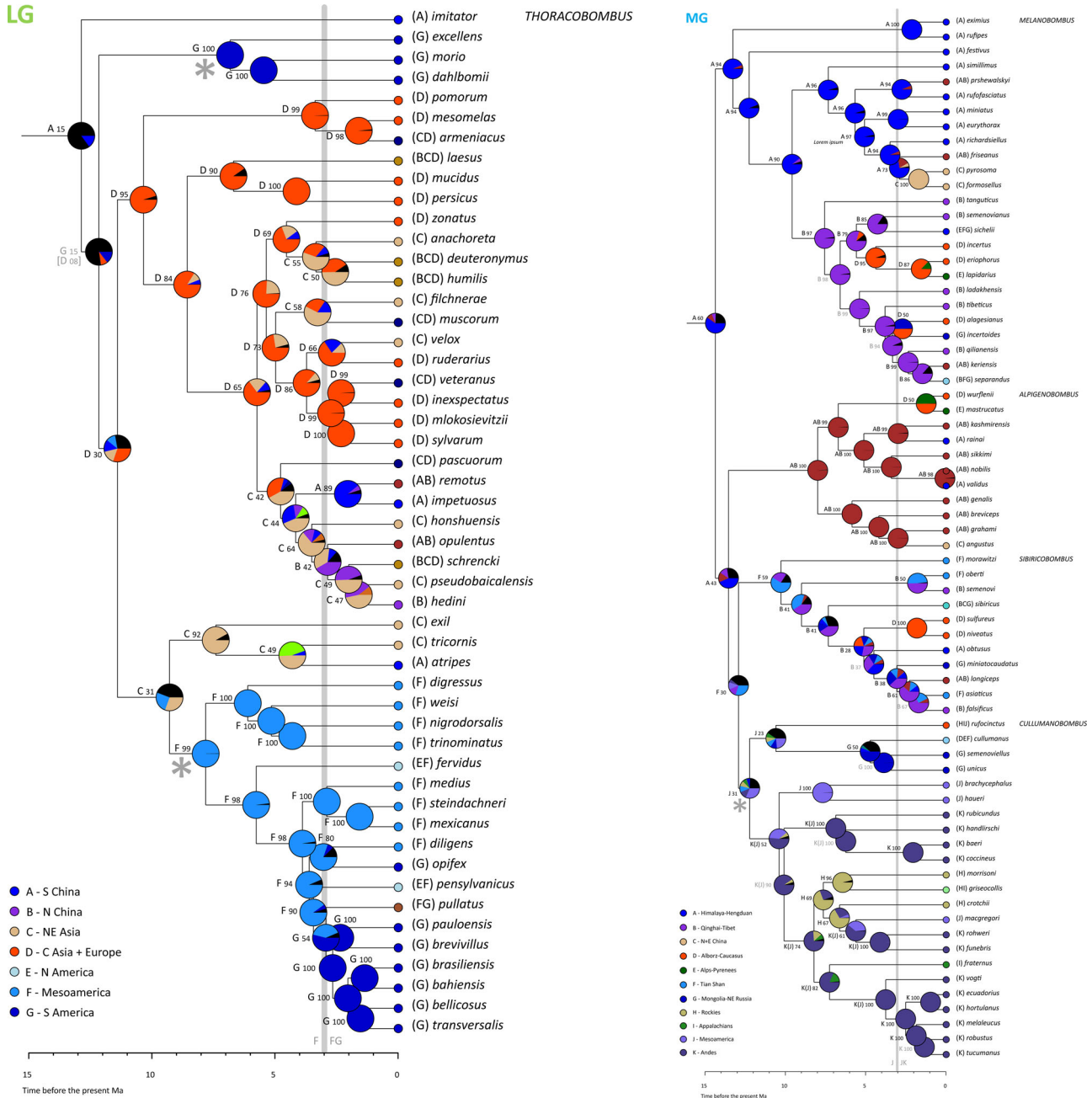
Areas that are especially frequent within ancestral area estimates for those LG lineages with many extant species (Fig. 13) include: Europe and Central Asia (area D) and north-eastern Asia (C) for the subgroups *pomorum*–*armeniacus*, *laesus*–*persicus*, and *zonatus*–*sylvarum*; north-eastern Asia (area C) especially for the subgroups *pascuorum*–*hedini* and *exil*–*atripes*; southern China (area A) for the subgroup *remotus*–*impetuosus*; and Mesoamerica (area F) and South America (area G) for the subgroup *digressus*–*transversalis*.

Areas that are especially frequent within ancestral area estimates for those MG lineages with many extant species (Fig. 14) include: the Himalaya-Hengduan area (area A) for much of the subgenus *Melanobombus* (except for the subgroup *tanguticus*–*separandus*, which is associated more with Tibet (area B) and then with the Alborz-Caucasus area (area D)), and the Himalaya and Tibet together (area AB) for the subgenus *Alpigenobombus*. In contrast, the area most frequent in ancestral area estimates for the subgenus *Sibiricobombus* is Central Asia centred on the Tian Shan mountains (area F) with extant descendent lineages mostly associated with Tibet (area B).

The larger South American groups with extant distributions endemic to South America (subgroups in the lower part of each of Figs 13–14) had a most likely estimated ancestral distribution in Mesoamerica (area F) for the LG subgroup *digressus*–*transversalis* and estimated for South America (area K) for the MG subgroup *brachycephalus*–*tucumanus* (NB with the dispersal models in Figs 2–3 entirely open in all time periods).

## Discussion Uncertainty

The estimated phylogenetic trees, dates, and biogeographic solutions in our results agree well with those from previous studies of these bumblebees (Cameron et al., 2007; Hines, 2008; Kawakita et al., 2004; Santos-Júnior et al., 2022; Williams, 1985). Re-analysis of the Cameron et al. (2007) five-gene data (without COI) using our methods gave nearly the same topology as obtained by them among all of the current subgenera as well as for the former subgenera within the larger,



**Figs 13, 14.** Most likely ancestral ranges reconstructed for all extant currently recognized species of (13) the lowland grassland (LG) group from the dispersal model in Fig. 2 and of (14) the montane grassland (MG) group from the dispersal model in Fig. 3, using the procedure DIVALIKE + J in S-BioGeoBEARS with samples of 10,000 trees from BEAST from the six genes in Figs 11–12. Letters represent the area units in Figs 2 and 3 respectively: letter combinations at terminals show updated species’ current distributions (keys lower left show the colour codes used for the principal areas of endemism, other colours are for combinations of areas, black is for other minority combinations of areas); letter combinations at nodes show the most likely reconstructions for ancestral distributions (where estimated South American ancestral distributions (G/K) pre-date the land bridge, likely Mesoamerican distributions are shown in parentheses (F/J)); numbers at nodes show the percentage of solutions in which the most likely solution appears (use of letters and colours for nodes to identify area combinations is not consistent between the two analyses; the numbers are shown in grey where posterior support for the node is <0.66); pies at nodes indicate the percentage of solutions for that node in which particular solutions occur. The grey stars indicate the arrival of lineages in Mesoamerica and the vertical grey bar shows 3 Ma. Numbers on the x-axis are ages in Ma before present. Outgroups have been removed (LG, *B. chinensis*; MG, *B. ignitus*).

currently accepted subgenera *Thoracobombus* and *Cullumanobombus*.

The data from the COI gene helped place the many newly added species as terminals on the LG and MG trees. There is no evidence for ancient numts in these COI data, which would have appeared as branches clustered near the root of COI-based trees, which have the potential to give a severe misrepresentation of relationships (Magnacca & Brown, 2010; Song et al., 2014) in the combined-gene trees. The placement of species within our trees is in broad agreement with what is expected from morphology (Williams, 1998a), with recent subgeneric revisions (*op. cit.*), and with recent recognition of cryptic diversity within the subgenera *Sibiricobombus* and *Alpigenobombus* (Williams, 2022).

An interesting case is *B. brevivillus*: its position as the sister species to *B. pauloensis* in Fig. 11 is unexpected, because Franklin (1913) had suggested when describing this species from the type that *B. brevivillus* is most similar to *B. mexicanus* Cresson in morphology (which we can support from comparing the female type of *B. brevivillus*: the female morphology is similar to *B. mexicanus* in (1) unusually short tibial fringes; (2) the flattened central area of the clypeus; and similar male genitalia: with (3) the volsella long and narrow; (4) the gonostylus with a reduced outer distal lobe; and (5) the penis and gonocoxa with enlarged bases). A thorough revision of the subgenus *Thoracobombus* throughout its range might illuminate this apparent conflict between DNA and morphological evidence. However, the biogeographic inferences here are unaffected by these uncertainties.

Results from Cameron et al. (2007), Sun et al. (2021), and our results when including COI (Fig. 12) all show highest uncertainty concerning the relationships of the subgenus *Alpigenobombus*. In addition, the species *B. imitator* is a ‘rogue taxon’ within the LG group depending on the outgroup used. Neither of these uncertainties has consequences for the biogeographic inferences here.

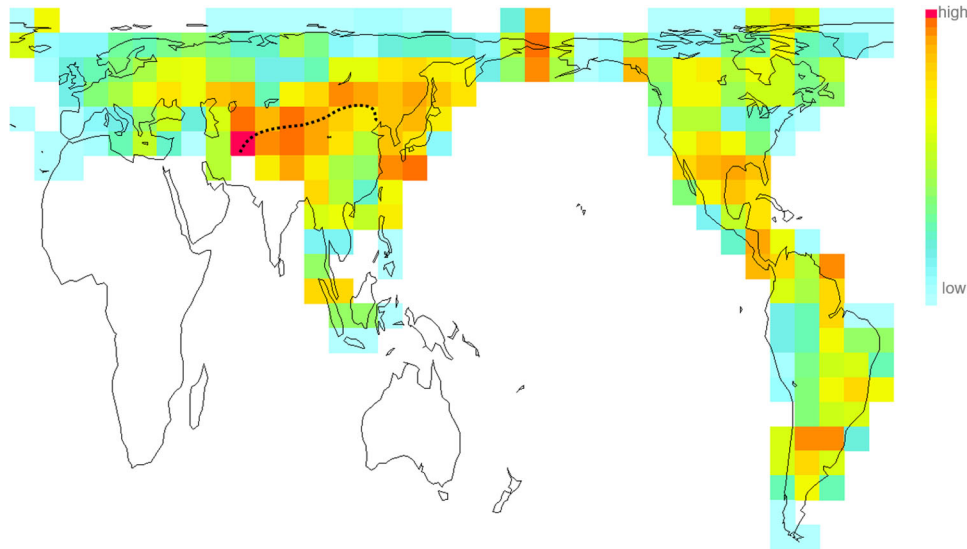
The most important uncertainty for this analysis is in the date estimate, which have many potential sources of error (Baum & Smith, 2012; Drummond & Bouckaert, 2015). Our date estimates are most affected by the crown-date calibration for the genus *Bombus*, which has been examined in detail by Hines (2008). Fortunately, for the questions addressed here concerning the dates of divergence for the South American species of the LG and MG groups, the level of precision required is low because only approximate relative estimates are sufficient. The key date for the most recent common ancestor (MRCA) of the MG group of South American bumblebees according to Hines (2008) was *c.* 11.9 Ma.

This event is estimated as *c.* 10.3 ( $\pm 2.8$ ) Ma in our Fig. 12, so both dates agree in being substantially earlier than the 3 Ma suggested for the opening of the South American land bridge (O’Dea et al., 2016).

### Early biogeography of bumblebees

All quantitative estimates of phylogenetic trees encompassing a broad representation of bumblebees have agreed that the two oldest sister lineages among all extant bumblebees are likely to be: (1) the MRCA of the subgenus *Mendacibombus*; and (2) the MRCA of all other extant bumblebees (Cameron et al., 2007; Kawakita et al., 2004; Santos-Júnior et al., 2022; Sun et al., 2021; Williams, 1985). Williams et al. (2018: their fig. 6a) argued that these two oldest extant sister lineages are likely to have had vicariant distributions between the mountains on either side of the Dzungar – Taklamakan – Gobi Desert belt that spans from Central Asia and Xinjiang through southern Mongolia to north-eastern China (with *Mendacibombus* to the south). This is an arid zone that developed especially with the Early Oligocene climatic cooling (Zachos et al., 2008) and remains the principal boundary between the large Palearctic (northern) and Oriental (southern) bumblebee faunas in Asia today (Williams, 1996). Only Santos-Júnior et al. (2022) place the first divergence between extant bumblebees substantially before the end of the Eocene. An updated map of species’ (spatial) turnover (Fig. 15) shows that this region is still the largest and most pronounced transition zone even for the revised bumblebee faunal species composition worldwide. It is most pronounced in the west (around Kashmir) and slightly weaker in the east (the Khingan mountains of Neimenggu and Heilongjiang). The persistent barrier of severe aridity across most of Asia appears to have had a major influence throughout bumblebee evolution, presumably because the typically limited rainfall and flowering regimes in these areas would be severely unfavourable to the long activity seasons of most bumblebee colonies.

The original northern group of bumblebees (the sister-group to the subgenus *Mendacibombus*) includes early-diverging extant lineages that currently have broad steppe distributions in Asia as *B. confusus* Schenck and *B. soroensis* (Fabricius) (Williams et al., 2018: in Fig. 1 these are the subgenera *Bombias* and *Kallobombus*, which include just four species). Almost all of the other extant species descended from the northern group belong to the two sister lineages: the LF bumblebees and SF bumblebees (Cameron et al., 2007), distributed primarily in the lowlands (Fig. 5: LF) or in the mountains (Fig. 6: SF) respectively. These groups are



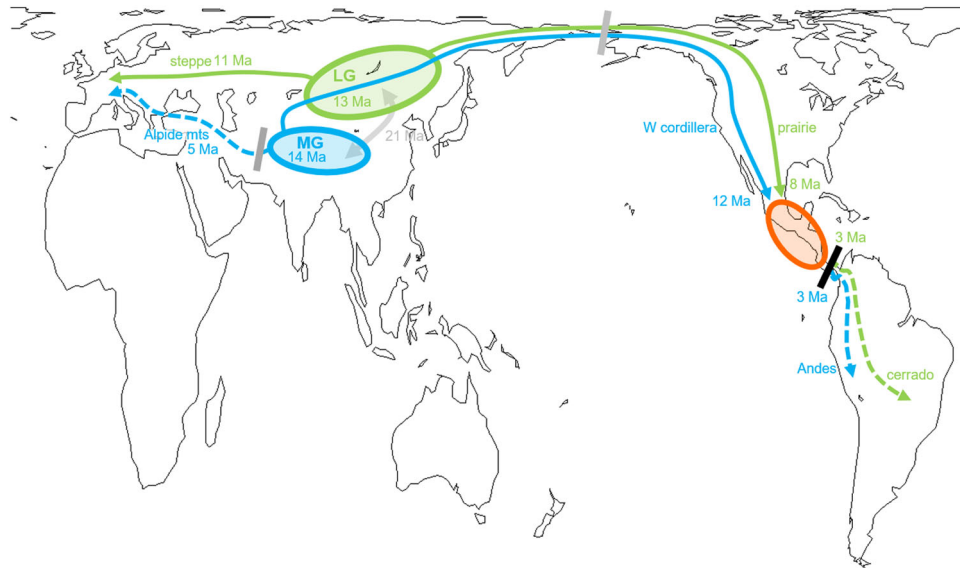
**Fig. 15.** Variation in the strength and breadth of transition zones between faunal regions for all bumblebee species, mapped for the updated data in Fig. 4 with the  $\beta$ -3 measure of species' turnover by replacement between any pairs of species within a 9-cell moving-window neighbourhood (Williams, 1996; Williams *et al.*, 1999). Dark orange shows strong boundaries with many species' replacements among neighbouring cells, whereas blue shows areas with few species' replacements among neighbouring cells. The black dotted line traces the middle of the principal Asian arid barrier. The map and grid are as shown in Figs 4–10.

represented in our analysis by the lowland grassland group (Fig. 7: LG) and by the montane grassland group (Fig. 8: MG). Extant LG bumblebees are the most likely to nest among dense grass on the surface of the ground in open grassland plains and these species include the 'carder' bumblebees (Sladen, 1912; although they do sometimes nest in cavities below ground). In contrast, the MG bumblebees most often nest in cavities underground or in enclosed or covered cavities above ground, often in more protected situations among rocks (Sakagami, 1976; Williams *et al.*, 2008). There are exceptions to these habitat associations: the LG group includes a minority of montane species in the south-European, Hengduan, and Andes mountains (e.g. *B. mucidus* Gerstaecker, *B. impetuosus* Smith, and *B. excellens* Smith respectively); and the MG group includes a few species that extend outwards from mountains into the lowlands in Asia, northern Europe, and North America (e.g. *B. sichelii* Radoszkowski, *B. lapidarius* (Linnaeus), and *B. griseocollis* (DeGeer) respectively). Nonetheless, among the descendants of the original early northern group, the LG ancestral lineage sister to the *morio*-group is estimated to have had a distribution centred on northern Eurasia (Figs 13, 16) and the MG ancestral lineage is estimated to have been centred in southern Asia (Figs 14, 16).

Bumblebees are likely to have reached Europe from Asia by two distinct paths: to the north or to the south of the Caspian Sea. To the north, for long periods when climate allowed (when not glaciated), there may have

been a grassland corridor for a diversity of primarily LG bumblebees to disperse from northern Asia into Europe (Figs 7, 16). There are no extant species of this group now in the western Himalaya and few in Central Asia. Climatic cooling and expanding grasslands after *c.* 10 Ma (Zachos *et al.*, 2008) may have contributed to the dispersal and divergence of many LG lineages with extant descendants in north-eastern Asia (area C) and Europe (area D) in Fig. 13. In the south, MG bumblebees dispersed westwards along the 'Alpide' mountain belt from Afghanistan to the Pyrenees, but only during a more restricted period when climates were cooler, wetter, and more favourable to montane bumblebees than they are now, probably between *c.* 8–3 Ma (Williams *et al.*, 2018: their fig. 7; Figs 8, 16). Figure 15 shows that a separation between faunas following these two paths is still marked by a broad transition zone in faunal composition (i.e. higher species' replacement) between the northern and southern faunas within eastern Europe and the Kazakh desert.

With the opportunities provided by the new land connections between Asia and North America after the end of the Oligocene (Sanmartin *et al.*, 2001), there is broad agreement that climatic fluctuations (Zachos *et al.*, 2008) are likely to have facilitated the dispersal of bumblebees via Beringia into North America (Hines, 2008; Kawakita *et al.*, 2004; Williams, 1985). It has been noted that this dispersal was not entirely one-way, with several lineages later returning to Asia via Beringia (Hines, 2008; Kawakita *et al.*, 2004; Williams, 1991).



**Fig. 16.** Summary of the dispersal history of the lowland grassland (LG, green) and montane grassland (MG, blue) groups of bumblebees from Asia into Europe, North America and South America, based on (see the text) the most likely reconstructed ancestral distributions from the S-BioGeoBEARS results in Figs 13–14. Approximate dates are from estimated ages of the oldest nodes (Figs 11–12) for lineages for which an area is part of the reconstructed ancestral distribution (Figs 13–14) allowing for availability of corridors. Principal centres of divergence among extant lineages are shown with ellipses for LG bumblebees in north-eastern Asia (green), for MG bumblebees in the Himalaya-Tibetan region (blue), and for both groups in Mesoamerica (orange). For simplicity, dispersal events in the reverse directions are not shown. Barriers to dispersal that are likely to have been removed during restricted periods are shown as grey bars (Afghanistan, 8–3 Ma: Williams et al., 2018; and Beringia: Williams et al., 2019) and a black bar (Panama, <3 Ma: O’Dea et al., 2016). The underlying map is as shown in Figs 4–10.

This has been supported by more focused studies of the subgenera *Alpinobombus* (Williams et al., 2019) and *Pyrobombus* (Martinet et al., 2019; Potapov et al., 2017; Sheffield et al., 2020). Moreover, the exceptional species’ radiation of the subgenus *Pyrobombus* within western North America is evidenced by high species richness there in Fig. 6. However, the only lineage within the LG and MG groups that is strongly supported (Fig. 14) as having returned to Asia (via Beringia) is the sister lineage to *B. rufocinctus* Cresson within the subgenus *Cullumanobombus*, which had dispersed back to Asia by *c.* 4.7 Ma. The *morio*-group must have shown a particularly early and dramatic dispersal after diverging from an Old World sister group, through North America and Mesoamerica, but with extirpation now of its entire lineage from those regions.

The uplift of the principal mountain systems of Mexico and Mesoamerica in the Late Cretaceous and Eocene (the Mexican Orogen) was already largely in place (Fitz-Diaz et al., 2018) by the time the MG-group bumblebees dispersed to Mesoamerica by *c.* 12.1 Ma (Fig. 14). The later establishment of a permanent land corridor at the isthmus between North and South America has long been believed to have occurred at *c.* 3.5–3 Ma (Coates & Obando, 1996). Such a late date for the land connection has been challenged recently

(Bacon et al., 2015). However, a more recent review of broad evidence overwhelmingly supports a date of *c.* 3 Ma, except for some species capable of being rafted across the ocean between islands or continents (O’Dea et al., 2016). This is unlikely to include bumblebees with their high energy demands and subterranean hibernation. The land bridge had been suggested to be necessary for bumblebees to disperse to South America (Williams, 1985; Kawakita et al., 2004). Figure 15 shows transition zones in contemporary faunal composition (high species’ replacement) across northern Mexico and across the Mesoamerican isthmus in the vicinity of Costa Rica. These boundaries surround a secondary hotspot of species’ richness and endemism in southern Mexico (Figs 4–10).

### The mystery of South America

The most surprising feature of Hines (2008) and Santos-Júnior et al.’s (2022) biogeographic reconstructions is the proposition that the first bumblebees may have dispersed from North America to South America perhaps as early as 17–7 Ma (in the Miocene Epoch, Hines’ fig. 7), even though Hines accepted that establishment of the permanent land corridor did not occur until 3 Ma (Pliocene Epoch). Hines suggested that this discrepancy



might be explained by earlier inter-continental dispersal via an island arc. The indigenous occurrence of bumblebees on Caribbean islands (which are of much older origin) has been discounted (Williams 2015). Because we conclude that there is generally little support for successful establishment by bumblebees following long-distance dispersal across seas (see the Introduction), the timing of the arrival of bumblebees in South America remains an open question.

### A tale of two habitats: late dispersal of LG bumblebees

The ancestral distribution estimated for the LG group is most likely to have been in northern Asia (Fig. 13: area A 15%), similar to the original northern sister group that was identified in the *Mendacibombus* analysis (Williams *et al.*, 2018: their fig. 6A). Much of the early pattern of divergence to extant lineages of the LG group seems to have taken place broadly between lineages with extant descendants now in the north-Eurasian region (Figs 2, 13: areas C and D), even if later glaciations may have left only relatively broadly distributed species in the north today (Figs 7, 9) (the group of *excellens-transversalis* is not well supported in Fig. 11 and, even if it were, dispersal of a daughter lineage to South America and back again in Fig. 13 [G in grey] is extremely unlikely). Two of the LG lineages appear to have dispersed to North America (Figs 2, 13: area E) and to Mesoamerica (area F) by *c.* 7.8 Ma (the subgroups *excellens-dahlbomii* and *digressus-transversalis*: Fig. 13, marked by asterisks). There are many divergences into extant lineages within the New World between *c.* 7.8–1.4 Ma (Fig. 13). This coincided with declining global temperatures (Zachos *et al.*, 2008) and an expansion of grasslands (Arakaki *et al.*, 2011). Five extant lineages of the LG group may later have been able to disperse across the new land bridge from Mesoamerica (Fig. 13: area F) into South America (area G) after *c.* 3 Ma. However, three of these five lineages are in the *morio*-group, currently the most cold-adapted LG bumblebees in South America, which in their cooler habitat associations are more like the MG group (discussed below). For the remaining LG species, the well-supported MRCA of the predominantly South American subgroup *pullatus-transversalis* is dated at *c.* 2.9 Ma in Fig. 11, with the 95% highest posterior density interval for this age estimate extending to 2.0 Ma. Therefore this MRCA may have been able to disperse to South America via the emerging land bridge after *c.* 3 Ma. Subsequently, the lineages to both *B. fervidus* (Fabricius) and *B. pensylvanicus* (DeGeer) may have dispersed back northwards from Mesoamerica (where

there is currently the greatest genetic diversity for these two species complexes, which still need thorough revision) and into North America. The LG lineages are likely to have dispersed southwards through lowland or temperate grassland corridors, perhaps along the coasts of the isthmus. There was then rapid divergence of the lineage into the many extant species within South America (Fig. 13), with a current centre of endemism in south-eastern Brazil (Fig. 9). A range of techniques have been applied to explore the finer details within South America (Abrahamovich *et al.*, 2004; Franoso *et al.*, 2016; Santos-Junior *et al.*, 2015; Santos-Júnior *et al.*, 2022; Skorikov, 1923), but more detailed work is needed. Thus our interpretation of the history of the LG group in the New World agrees broadly with earlier studies that emphasize the dependence on the Pliocene land-bridge corridor for their arrival in South America (Kawakita *et al.*, 2004; Williams, 1985).

In contrast, the ancestral distribution of the MG group is likely to have been further to the south in Asia, in the high mountains of the Himalaya-Tibetan region (Figs 3, 14: areas A and B, 16). A difference in elevation and climatic preferences between LG and MG bumblebees has been noted before (Williams, 1985; Kawakita *et al.*, 2004). Much of the early divergence of MG lineages with extant descendants appears to have occurred initially in this Himalaya-Tibetan region (e.g. the MRCAs of each of the subgenera *Melanobombus*, *Alpigenobombus*), with divergences in Central Asia for the lineages to the MRCAs of the subgenera *Sibiricobombus* and *Cullumanobombus*. The MRCA of *Cullumanobombus* with extant descendants is estimated in Fig. 14 (asterisk) to have dispersed (from Central Asia, area F) to Mesoamerica (area J) by *c.* 12.1 Ma. The many lineage-node distributions within *Cullumanobombus* estimated to have been present in South America (area K) after *c.* 10.3 ( $\pm 2.9$ ) Ma but distinctly before the land bridge that is believed to have been established at *c.* 3 Ma are then problematic.

### Resolution of the date mismatch: delayed dispersal of MG bumblebees?

However, if we accept the early estimated dates (from *c.* 10.3–3.0 Ma) for divergence among the many MG group lineages with ancestral distributions estimated to be in South America (Fig. 14: area K), then there is nonetheless a simple explanation that is consistent with there being a complete barrier to bumblebees dispersing to South America before *c.* 3 Ma. It could be that these MG lineages diverged within the mountains of Mesoamerica (area J) before the land bridge (JK) was established at *c.* 3 Ma and that they later dispersed to

South America only once the land bridge was established, perhaps when climates were also cooler. Once corridors of suitable montane grassland habitat were available after *c.* 3 Ma, at least seven of these lineages might have dispersed southwards into South America (Figs 14, 16). All except one of these seven lineages would have to have been extirpated subsequently within Mesoamerica (erasing direct evidence of their former distributions in area J, just as must also be required for the lineage to the *morio*-group), perhaps because the Mesoamerican mountains were too low and the interglacial periods were too warm and variable (cf. Dirnböck et al., 2010). Some of the post 10.3 Ma but less extreme montane lineages may have dispersed back northwards from Mesoamerica into North America (Figs 3, 14: area H): as the extant subgroups of *morrisoni-griseocollis*, *crotchii*, and *fraternus*. The other South American lineages may not have followed northwards because of a developing barrier of increasing aridity in the Sonoran and Chihuahuan deserts of northern Mexico during this period, which may have cut off corridors of habitat suitable for their northward dispersal (Arakaki et al., 2011; Hernandez-Hernandez et al., 2014). A comparable pattern of increased diversification *c.* 15–5 Ma in Mesoamerica and especially in Mexico is reported for the tree genus *Pinus*, which is also associated with mountains in Mexico (Jin et al., 2021). *Pinus* has its centre of greatest species richness worldwide in southern Mexico and the species there are especially young (Jin et al., 2021). However, many of those *Pinus* species now have unusually narrow and highly fragmented geographic distributions in the Mexican mountains (Critchfield & Little, 1966), which is consistent with range contractions following increasing aridity and the effect of climate cycles on small montane ranges.

The many MG node distributions estimated to have been present in South America (Fig. 14: area K) before the land bridge existed are only a parsimonious solution for the S-BioGeoBEARS method because the dispersal model in Fig. 3 could be misleading, to the extent that the corridor joining areas JK may have been unavailable until after *c.* 3 Ma. Therefore, earlier estimates of ancestral distributions in area K would actually have been in area J (even if they were subsequently lost from there). With this solution fitting the date and distribution estimates, this interpretation of the history of the MG group in the New World would then agree with the earlier biogeographic studies for bumblebees (Williams, 1985; Kawakita et al., 2004), if not with Hines (2008) and Santos-Júnior et al. (2022).

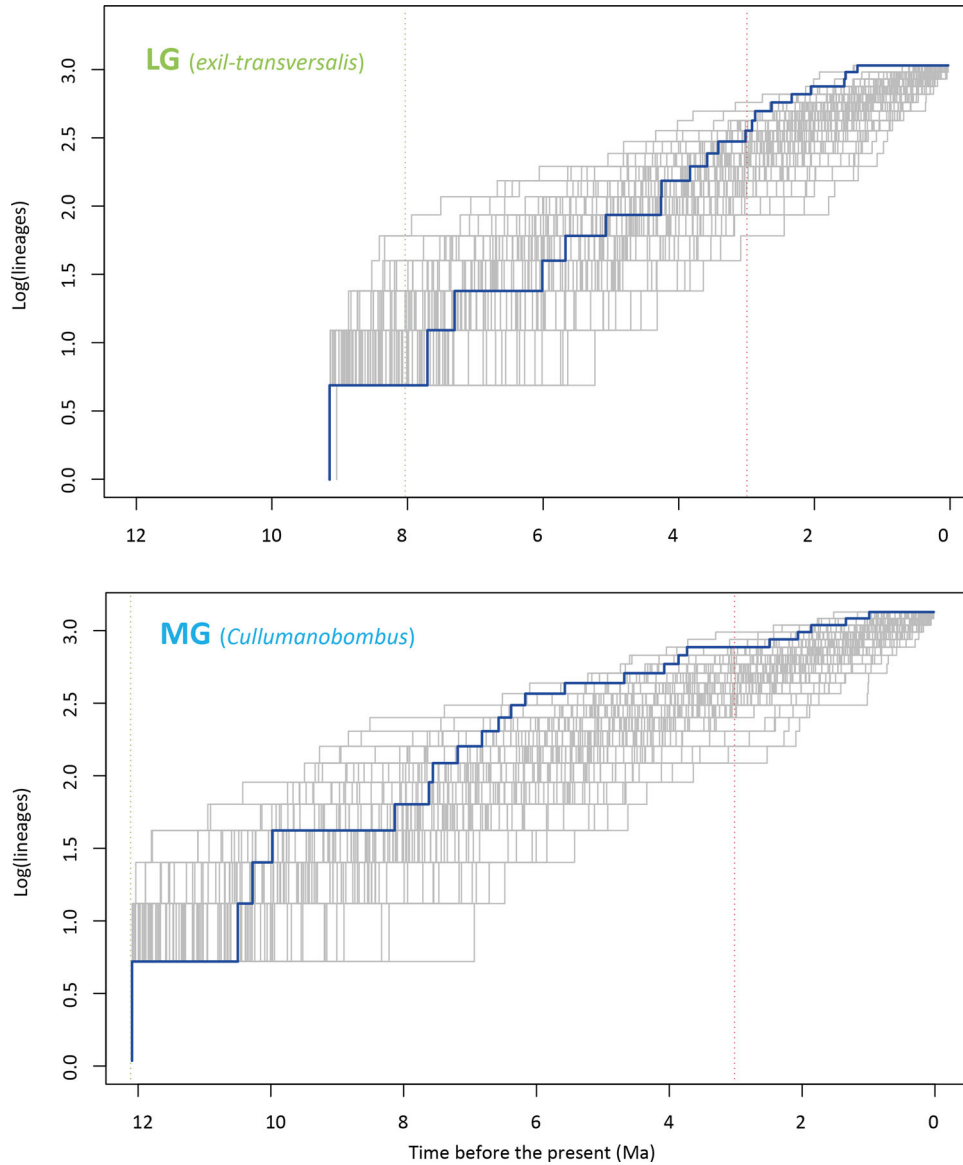
Rates of divergence into new lineages in Figs 11 and 12 can be compared in lineage-through-time plots (Budd & Mann, 2018) in Figs 17 and 18, all the more easily

because these trees include all of the known and accepted extant species in these groups. In each case the plot is restricted to a large monophyletic group that includes all of the New World species and excludes other groups of Old World species. The grey lines represent 100 simulations of a simple pure-birth process for these trees (using the PHYTOOLS *R* package: Revell, 2012), so when the observed shape of the trees (blue) is within five (grey) lines of the edge of this envelope (single-tailed test) it shows that this tree is more extreme than expected. Therefore our trees show an unexpected excess of new lineages after 3 Ma (after the red dotted line) for both groups, although this departure begins much earlier (*c.* 6.5 Ma) for the MG group. These results are silent on the location of where the divergences occurred within the New World, but they are consistent with proposed radiations of LG lineages in South America after 3 Ma, and of MG lineages in Mesoamerica after 6.5 Ma that subsequently continued into South America. The plot could in principle show any possible complete extinction of lineages as lower values for observed numbers of lineages compared with the pure birth model, but cannot show any of the regional extirpations that are postulated here for the parts of populations within Mesoamerica for MG lineages. While it might be tempting to see in these plots some ‘pull of the present’ (Budd & Mann, 2018), neither group shows strong evidence of complete extinction of many lineages as cryptic mass extinctions (Crisp & Cook, 2009). Significantly, they do support an increase in recently diverging extant lineages as having started earlier for the MG group than for the LG group as proposed here.

Why does there appear to be a substantial difference between the LG and MG groups in their temporal patterns of divergence to extant species in Mesoamerica and South America? This may be explained in part by the earlier arrival of the MG lineage than the LG lineage in Mesoamerica, giving more time to accumulate speciation events and more lineages there (Fig. 16). In addition, the complexity of the mountains in this region combined with climatic fluctuations may have caused a higher speciation rate for the montane MG lineages (with a variety of local refuges) compared with the lowland LG lineages. Future work to model past habitat suitability with changing climates during this period may help to answer this question.

### A mammoth step for conservation

During the Anthropocene, the northern steppe and prairie grassland habitat of the LG bumblebees in Europe, Asia, and North America has been under increasing



**Figs 17–18.** Log-transformed counts of the number of lineages through time (in blue) plotted against time before the present (in Ma) for (17) the LG subgroup *imitator-transversalis* in Fig. 11 and (18) the MG subgenus *Cullumanobombus* in Fig. 12. The grey lines represent 100 simulations of a simple pure-birth process for these trees. The green dotted line (left) represents the estimated date of arrival of each of these groups in Mesoamerica and the red dotted line (right) indicates 3 Ma estimated for the land bridge to South America.

pressure for conversion to cereal farming. This threatens especially the LG bumblebees, not only through the direct destruction of their food plants, but also indirectly through the use in these agricultural areas of insecticides, herbicides, and fertilizers (Arbetman *et al.*, 2017; Cameron & Sadd, 2020; Williams & Osborne, 2009). This may be seen particularly in the dramatic range reductions of some of the once more widespread Old World specialist steppe bumblebees, *B. cullumanus* (Kirby) (Williams *et al.*, 2013) and *B. fragrans* (Pallas) (Williams *et al.*, 2011).

In some periods before the Anthropocene, the northern grasslands may have been even more extensive. Grasslands often occur where succession to forest is prevented, either because of low water availability (in arctic, alpine, and warm semi-arid environments) and/or where trees have been felled by fire or by large-mammal browsers (it has been claimed that some savannahs in Africa are maintained by elephants pushing over trees to feed: Bond, 1994). During the last 100 ka, a very extensive northern ‘mammoth steppe’ in Europe, Asia, and North America resulted from dry conditions and

perhaps from the tree-felling behaviour of large herbivorous mammals (Guthrie, 1982) and this is very likely to have benefitted bumblebees. Conservation of the many threatened LG bumblebees (Arbetman et al., 2017) would certainly be helped by re-establishing larger areas of flower-rich tall grassland.

## Conclusion

Our results do not prove that bumblebee dispersal was long delayed in Mesoamerica before finally reaching South America via the Panamanian land corridor after c. 3 Ma. But we do show that this explanation is consistent with more of the premises, for: (1) our dated species' trees; (2) the best supported date of the land bridge; and (3) what is currently understood of the limitations to bumblebee long-distance dispersal and establishment. By considering the history of dispersal of lowland and montane grassland bumblebees separately, we can then explain more precisely how some of the now endemic South American bumblebee species have estimated dates of divergence from long before the land connection with Mesoamerica was established. We propose that the montane grassland bumblebees arrived earlier in Mesoamerica, and that the majority of the extant South American MG lineages may have diverged within the Mesoamerican mountains over a period of up to 9 Myr before the land connection southwards arose; whereas the lowland grassland bumblebees arrived in Mesoamerica much later, and that the majority of the extant South American LG lineages may have diverged within South America and only after the land connection had formed.

The present study reinforces the need for dynamic habitat models in biogeographic analyses, demonstrating that consistent explanations may depend on incorporating (1) the diverging habitat preferences as well as (2) the changing availability of suitable habitat connections. This supports a previous analysis for *Mendacibombus* bumblebees that had shown that dispersal models may need to change through time, accommodating (for example) not only evolving habitat requirements in terms of changing thermal tolerances along lineages, but also a changing distribution of suitable habitat as regional climates change (Williams et al., 2018). Complete and thorough global taxonomic revisions are still needed for the subgenera *Thoracobombus* and *Cullumanobombus*, demonstrating a clear need for taxonomy as a basis for evolutionary investigations.

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Design, analysis, and writing by PW; DNA sequencing and discussion of the final manuscript by all co-authors.

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