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RESEARCH ARTICLE

Conservation genetics of European bees: new insights from the continental scale

Thomas Lecocq[1,2] · **Maxence Gérard**[2] · **Denis Michez**[2] · **Simon Dellicour**[3]

Abstract Population connectivity is an important source of information for planning conservation strategy. The degree of connectivity implies using alternative conservation prioritizations based on the appropriate spatial scale for management units. In species with low population connectivity, it is important to preserve local populations in order to maintain the species throughout its range. In contrast, species with high connectivity require extensive management aiming at preserving gene flow through the entire species range. Here we examine at the continental scale the relationship between inter-population/inter-individual genetic and geographical distances to study the isolation-by-distance (IBD) pattern within the European range of nine wild bee species. We then assess the suitability of multi-local or extensive conservation to ensure their long-term survival. Results based on inter-population differences show only two out of nine species with significant IBD. European bee populations seem quite connected when their IBD is compared to IBD of other phyla. However, our results based on inter-individual distances show that eight out of nine species display significant IBD. These different results are presumably a consequence of potential limitations of the inter-population approach. Therefore, we speculate that the inter-population approach could result in inaccurate IBD estimations. This approach should therefore be replaced by the inter-individual approach in order to provide strong supported conservation guidelines. We support multi-local conservation programs based on our analysis of inter-individual distances.

Keywords Conservation · Europe · Genetic differentiation · Isolation by distance · Wild bees

✉ Thomas Lecocq
thomas.lecocq@univ-lorraine.fr

✉ Simon Dellicour
simon.dellicour@kuleuven.be

1 Research Unit Animal and Functionalities of Animal Products (URAFPA), University of Lorraine INRA, 2 Avenue de la Forêt de Haye, BP 172, 54505 Vandœuvre-lès-Nancy Cedex 09, France

2 Laboratory of Zoology, Research Institute of Biosciences, University of Mons, Place du Parc 20, 7000 Mons, Belgium

3 Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, KU Leuven—University of Leuven, Herestraat 49, 3000 Leuven, Belgium

Introduction

One of the most important source of information for conservation management is an accurate knowledge of the population connectivity (Morton et al. 2009; Sutherland et al. 2009; Kingsford et al. 2009; Pullin et al. 2009). Population connectivity integrates two phenomena acting at different temporal and spatial scales: (i) the movement of individuals between adjacent populations and residence for enough time to contribute to the gene pool of the receiving populations (i.e. migration; Frankham et al. 2010) and (ii) the species range-wide gene flow (Broquet and Petit 2009). The former mainly relies on individual migration capacity while the latter may be borne by successive generations and thus exceeds individual migration capacity (Broquet and Petit 2009). The two phenomena are intrinsically linked since individual migration movements shape neighboring

population connectivity, which is a component of connectivity within the entire species distribution.

Most conservation studies consider individual migration capacity at a local scale to estimate population viability, reverse habitat fragmentation, and facilitate metapopulation dynamics in a particular region (Sutherland et al. 2009; Pullin et al. 2009; Doerr et al. 2011; Driscoll et al. 2014). However, an assessment at large geographic scale (i.e. continental level) of species range-wide gene flow could show if distant populations should be regarded as distinct entities and whether or not gene flow occurs throughout the species range. This can provide useful baseline information prior to further local conservation plans. Indeed, delineating the overall connectivity between populations allows assessing whether and how allopatric populations are mutually supportive, reinforcing, and evolving together (Rocha et al. 2007). This aspect represents an important prior to appropriately distribute limited funding resources in order to maximize population resilience and minimize species' risk of extinction (Frankham et al. 2010). Moreover, the persistence over decades of a species within its entire distribution should remain the goal to strive for, especially for organisms providing ecosystem services (i.e. benefits that humans derive from ecosystems). The long-range population connectivity can provide such a knowledge on whether the species is likely to persist in the long-term throughout its distribution (Gadgil 1971; Frankham et al. 2010). Indeed, a high range-wide connectivity maximizes rescue effect between distant regions and minimizes the genetic drift hazard for distant regressing populations while low connectivity increases the regional extinction risk and subsequently the species endangerment (Taylor and Dizon 1999; Frankham et al. 2010). From a conservation point of view, the range-wide connectivity pattern of a species should inform conservation prioritizations based on an appropriate spatial scale for management (Palsbøll et al. 2007). For species with low range-wide connectivity, conservation plan should consider distant populations as distinct and possibly artificially manipulated gene flow (Frankham et al. 2010). Therefore, spatially prioritized conservation actions (Margules and Pressey 2000) focusing on key population groups poorly connected with others (i.e. multi-local conservation plans) are better suited for such species. In contrast, spatially prioritized conservation strategies for species with high-connectivity throughout their distribution could be suboptimal as regards both the effort and the use of funds compared to an extensive management aiming at preserving current gene flow through the entire species range (i.e. an extensive conservation plan).

Since the range-wide connectivity is a key feature for designing conservation management, there is a premium to estimate it. Commonly used methods to assess the individual migration capacity, such as parentage analyses or genetic tagging (Driscoll et al. 2014), provide short-term and short-range estimates of connectivity which are useful at local scale but unsuitable for estimating connectivity within the entire species range (i.e. species range-wide gene flow) (Avise 2000; Lowe and Allendorf 2010). In contrast, methods based on genetic distances (Rousset 1997, 2000) integrate the dispersal over many generations, which exceeds the distance that any one individual could achieve (Broquet and Petit 2009) and thus reflects the species range-wide gene flow. Spatial patterns of genetic distances between populations (Rousset 1997) or individuals (Rousset 2000) are increasingly used to estimate this overall connectivity by evaluating isolation-by-distance (IBD) (Banks and Peakall 2012; Lotterhos 2012). The IBD population genetic model assumes that genetic distance between individuals or populations increases with geographic distance (Rousset 1997). Such model allows estimating the mean dispersal averaged over many recent generations (being less affected by rare dispersal events) and thus the within species connectivity without the need to obtain data on prior population structure (Rousset 1997). The IBD model is frequently used in conservation biology to infer the connectivity extent among populations: the lack of IBD signal could be interpreted as a very high range-wide gene flow or as a very low gene flow even over short distances (review in Crispo and Hendry 2005). However, multiple species comparison of IBD patterns are still lacking for many phyla, and the present paper explores the IBD pattern for European bees (Hymenoptera, Anthophila).

Bees are the main pollinators of flowering plants in many natural habitats and agricultural crops (Ollerton et al. 2011; Kleijn et al. 2015). The current decline of bee populations raises worries that a major ecosystem service might be disrupted (Williams and Osborne 2009; Winfree 2010; Vanbergen 2013; Nieto et al. 2014; Goulson et al. 2015; Lecocq et al. 2016b). Several conservation actions and research programs have been developed to counter this alarming phenomenon, mainly at regional or national scale (e.g. Department for Environment, Food and Rural Affairs 2014) but increasingly at a continental scale (Potts et al. 2011). However, assessment of species range-wide gene flow is still lacking. Yet, it could allow rationalizing bee conservation across very large geographic areas (e.g. continental level). It should now be possible to fill this knowledge gap for European wild bees, following several recent continental-level genetic studies (Lecocq et al. 2013a, b, 2015a, b, c, 2016a; Dellicour et al. 2015a, b). These studies underline intraspecific differentiation within the species ranges, so one could expect IBD processes and subsequently low connectivity between these European bee populations. A first multi-species IBD assessment in European taxa would be a first step toward generalizing IBD patterns to non-European

bees. Yet, common pattern observed in co-distributed closely related species could be shaped by bee-specific or European-specific demographic dynamics. Therefore, generalization attempt should consider more bee taxa (including non-European species) as well as non-bee species to be efficient in order to evaluate if common IBD patterns are taxonomic or geographic-dependent.

In this study, we evaluate the species range-wide connectivity in wild bee species through analyses of their isolation-by-distance (IBD) pattern across Europe. We focus on nine social or solitary bees co-distributed in Europe. We investigate both their inter-population and inter-individual IBD patterns. Then, we compare our nine bee species to other non-bee European species and other bee species in order to assess the potential suitability of multi-local or extensive conservation to ensure the long-term species survival.

Materials and methods

DNA datasets

We considered nine European wild bee species: three solitary bees of the genus *Melitta* (Melittidae) and six eusocial species of the genus *Bombus* (Apidae): *Melitta leporina* (n=128 individuals, p=39 sampled localities/populations), *Melitta nigricans* (n=164, p=38), *Melitta tricincta* (n=98, p=23), *Bombus gerstaeckeri* (n=71, p=11), *Bombus hortorum* (n=58, p=25), *Bombus lapidarius* (n=236, p=50), *Bombus pascuorum* (n=272, p=54), *Bombus pratorum* (n=109, p=60), and *Bombus terrestris* (n=105, p=18). Genetic datasets of each species have been previously published (Lecocq et al. 2013a, b, 2015a, b, c, 2016a; Dellicour et al. 2014, 2015a, b) (Online Resource 1). Selected specimens were all European (i.e. from European countries, the European part of Russia, and Turkey) and mainly males (98%, see original publications for more details). We defined a population as a group of specimens sampled in the locality (Online Resources 1–2). Genetic information based on five genes was available for *Melitta* species: mitochondrial gene COI (cytochrome c oxidase subunit I; ~900 bp) and four protein-coding nuclear genes, WgL (wingless; ~400 bp), NaK (sodium–potassium adenosine triphosphatase; ~750 bp), RNAp (RNA polymerase II; ~850 bp) and Opsin (long-wavelength rhodopsin; ~850 bp). Three genes constituted the *Bombus* datasets: COI (~1000 bp) and two protein-coding nuclear genes, EF-1α (elongation factor-1 alpha, F2 copy; ~800 bp), and PEPCK (phosphoenolpyruvate carboxykinase; ~900 bp). When some samples displayed less base pairs than others, we coded missing base pairs as missing values.

Isolation-by-distance patterns

We analyzed (i) COI individually, (ii) all nuclear genes combined, and (iii) all genes combined. We examined the relationship between genetic and geographical distances to investigate the IBD pattern of each species (Rousset 1997, 2000). In particular, we estimated the linear regressions between genetic distance estimated for each pair of sampled populations or individuals and the geographic distance between them. We thus used two alternative genetic distances: one inter-population and one inter-individual distance metric. The inter-population approach is the former and most commonly used approach, whereas the inter-individual method has been recently suggested as more efficient for continuously distributed species (Lotterhos 2012).

For the inter-population distances, we estimated pairwise $\Phi_{ST}/(1-\Phi_{ST})$ (Φ_{ST} *sensu* Excoffier et al. 1992; a statistic taking both allelic frequencies and DNA sequence mismatches into account) for COI and over all loci (all nuclear genes or all genes combined) using multi-locus weighted averages (Weir and Cockerham 1984). We noted that the pairwise Φ_{ST} value was not estimable for some pairs of populations due to a too small number of sampled individuals per population. For the combined dataset, 36 out of 741 pairwise values were not estimable for *M. leporina*, 27/703 for *M. nigricans*, 14/253 for *M. tricincta*, 1/55 for *B. gerstaeckeri*, 139/300 for *B. hortorum*, 14/1225 for *B. lapidarius*, and 838/1653 for *B. pratorum*. For the COI-based dataset, 92 out of 741 pairwise values were not estimable for *M. leporina*, 98/703 for *M. nigricans*, 59/253 for *M. tricincta*, 4/55 for *B. gerstaeckeri*, 139/300 for *B. hortorum*, 106/1225 for *B. lapidarius*, 12/1431 for *B. pascuorum*, 844/1653 for *B. pratorum* and 11/153 for *B. terrestris*. Finally, for the datasets with all nuclear genes combined, 15 out of 630 pairwise values were not estimable for *M. leporina*, 38/630 for *M. nigricans*, 15/231 for *M. tricincta*, 10/55 for *B. gerstaeckeri*, 268/276 for *B. hortorum*, 25/1225 for *B. lapidarius*, 21/1431 for *B. pascuorum*, 240/903 for *B. pratorum* and 2/153 for *B. terrestris*.

For inter-individual distances, we estimated pairwise multi-locus IID ("inter-individual distance"), a distance metric that is also based on both allelic frequencies and DNA sequence mismatches; ("IID2" as defined in Dellicour and Mardulyn 2014). We used SPADS 1.0 (Dellicour and Mardulyn 2014) to estimate pairwise Φ_{ST} and IID values.

For both the inter-population and inter-individual distances, we used the R-package "fields" (Fields Development Team 2006) in R (R Development Core Team 2013) to compute the great circle geographic distances (i.e. distances on the surface of the earth, here measured in kilometers) between each pair of populations/individuals. In addition to linear regressions (LR), we used R-package

"vegan" (Oksanen et al. 2011) in order to perform Mantel tests (Mantel 1967) based on 10,000 permutations.

Reviewing procedure

In order to detect common trend in bee species globally, we compared the IBD patterns of the nine target European species to those previously reported for other bee species in the world. We selected previous continental-level genetic studies on other bees available in the database Scopus. We selected four studies investigating a total of six species: five species from Neotropic ecozone with mitochondrial DNA sequences and one from Asia with ribosomal DNA sequences (Batalha-Filho et al. 2010; Duennes et al. 2012; Hines and Williams 2012; López-Uribe et al. 2014). For those datasets, we assessed IBD signals with the same statistical analyses than for the nine European bee species. Overall, our nine European species and these six taxa gather (i) eusocial bees (*Bombus* spp. and *Melipona quadrifasciata*) and solitary bees (*Melitta* spp. and *Eulaema* spp.), (ii) generalist pollen foragers (*Bombus* spp. and *Melipona quadrifasciata*) and specialist pollen foragers (*Melitta* spp. and *Eulaema* spp.), (iii) large size species (*Bombus* spp. and *Eulaema* spp.) and medium size species (e.g. *Melitta* spp.). This allows a comparison of IBD between groups of species with specific morphology and ecology.

Beside bee species comparison, we compared the IBD patterns of bee species to the IBD patterns evaluated for other animal groups. We considered studies that focus on IBD signal assessment on a large geographic scale (i.e. continental level or large part of an ecozone) in inland Animalia species from the database Scopus. We selected studies by using the keywords "isolation-by-distance" associated with one of the five keywords "genetic variability", "conservation biology", "connectivity", "insects" or "bees". We considered studies based on allozymes, microsatellites or mitochondrial, ribosomal and/or nuclear DNA sequences, for IBD assessment whatever the IBD assessment method used (i.e. inter-population or inter-individual approaches). We selected 14 studies including 23 species and we looked at the LR determination coefficient (R^2) and Mantel test (Table 1). We then compared our results with the results from 14 selected studies.

Results

Analyses of the nine studied European bees

For analyses based on COI, significant positive IBD signals (i.e. Mantel test [MT] and/or LR R^2 p value <0.05) were detected for *B. hortorum* (by MT and LR R^2), *B. lapidarius* (only by LR R^2), *B. pratorum* (by MT and LR R^2),

M. leporina (by MT and LR R^2), and *M. nigricans* (by MT and LR R^2) in analyses based on inter-population distance (Table 2). Considering inter-individual distance, COI-based analyses showed significant positive IBD signals for all species (by MT and LR R^2 but only by LR R^2 for *M. nigricans*) except *B. terrestris*.

Analyses of nuclear-based datasets highlighted significant positive IBD signals for *B. pascuorum* (by MT and LR R^2), *B. pratorum* (only by LR R^2), *B. terrestris* (by MT and LR R^2), and *M. leporina* (by MT and LR R^2) when using inter-population distance. Analyses based on the same datasets but using inter-individual distance showed significant positive IBD signals for *B. gerstaeckeri* (by MT and LR R^2), *B. lapidarius* (only by MT), *B. pascuorum* (by MT and LR R^2), *B. pratorum* (only by LR R^2), *B. terrestris* (only by LR R^2), *M. leporina* (only by LR R^2), and *M. tricincta* (only by MT) (*M. nigricans* displayed a significant but negative LR coefficient value; Table 2).

For combined loci analyses, significant IBD positive signals were detected (i) for *B. pascuorum* by MT and LR R^2 based on inter-population distance and (ii) for most of the species by MT (all species except *B. terrestris* and *M. nigricans*) and LR R^2 (all species except *B. terrestris*) based on inter-individuals distance (Table 2). For all genetic datasets, the linear regressions always show low values for the determination coefficient and low correlation statistic values (Table 2).

Analyses of other bee species

All Neotropical species displayed significant IBD signals revealed by Mantel tests and significant LR R^2 values only in analyses based on inter-individual distance (Table 3). The analyses of Asian *B. trifasciatus* revealed significant IBD signals, but only with the inter-individual distance (Table 3).

Review of IBD patterns in other species

Our review of current literature showed that (i) few studies have assessed IBD signals over a large geographic area and that (ii) the population-based approach is the only one used at this large spatial scale (Table S1, Table 2). We did not find any study analyzing inter-individual distance at a continental scale. Most studies found a significant IBD between populations (16 species on a total of 23; Table 1).

Discussion

Divergent IBD patterns between loci

The IBD patterns of the nine European bee species are somewhat different between mitochondrial and nuclear

Table 1 Results of the review of papers focusing on IBD (isolation by distance) pattern assessment on large geographic scale (i.e. at continental-level or on a large part of ecozone) in inland species of Animalia in the database Scopus (accessed on the 16th March 2016)

Phyla (class)	Species	Area	Genetic information	Statistics	Number of populations	Number of individuals	IBD significance	References
Arthropoda (Insecta)	*Bemisia tabaci*	Asia and Oceania	STR	Pairwise squared genetic distance D_{AS}, Jin and Chakraborty (1994)	37	755	Yes	De Barro (2005)
Arthropoda (Insecta)	*Bombus bifarius**	USA	STR	D_{est}, Jost (2008)	33	506	Yes	Lozier et al. (2011)
Arthropoda (Insecta)	*Bombus bimaculatus**	USA	STR	D_{est}, Jost (2008)	34	448	No	Lozier et al. (2011)
Arthropoda (Insecta)	*Bombus ephippiatus**	Central America	mtDNA	Φ_{ST}, Excoffier et al. (1992)	17	86	See Table 3	Duennes et al. (2012)
Arthropoda (Insecta)	*Bombus impatiens**	USA	STR	D_{est}, Jost (2008)	33	596	Yes	Lozier et al. (2011)
Arthropoda (Insecta)	*Bombus occidentalis**	USA	STR	D_{est}, Jost (2008)	13	230	Yes	Lozier et al. (2011)
Arthropoda (Insecta)	*Bombus pensylvanicus**	USA	STR	D_{est}, Jost (2008)	25	302	No	Lozier et al. (2011)
Arthropoda (Insecta)	*Bombus trifasciatus**	Asia	rDNA	Φ_{ST}, Excoffier et al. (1992)	15	28	See Table 3	Hines and Williams (2012)
Arthropoda (Insecta)	*Bombus vosnesenskii**	USA	STR	D_{est}, Jost (2008)	16	319	No	Lozier et al. (2011)
Arthropoda (Insecta)	*Dendroctonus ponderosae*	USA	AFLP	F_{ST}, Weir and Cockerham (1984)	8	190	Yes	Mock et al. (2007)
Arthropoda (Insecta)	*Eulaema bombiformis*	South America	mtDNA	Φ_{ST}, Excoffier et al. (1992)	15	68	See Table 3	Lopez-Uribe et al. (2014)
Arthropoda (Insecta)	*Eulaema cingulata*	South America	mtDNA	Φ_{ST}, Excoffier et al. (1992)	22	90	See Table 3	Lopez-Uribe et al. (2014)
Arthropoda (Insecta)	*Eulaema meriana*	South America	mtDNA	Φ_{ST}, Excoffier et al. (1992)	20	145	See Table 3	Lopez-Uribe et al. (2014)
Arthropoda (Insecta)	*Melipona quadrifasciata**	South America	mtDNA	Φ_{ST}, Excoffier et al. (1992)	55	145	See Table 3	Batalha-Filho et al. (2010)
Arthropoda (Insecta)	*Polyommatus icarus*	Europe	Allozyme	Nei (1978) standard Genetic distance	29	1174	Yes	Schmitt et al. (2003)
Cordata (Actinopterygii)	*Anguilla anguilla*	Europe	Allozyme	F_{ST}, Weir and Cockerham (1984)	8	304	Yes	Maes and Volckaert (2002)
Cordata (Amphibia)	*Allobates femoralis*	South America	mtDNA	PAUP (Swofford 2001)	8	125	No	Amézquita et al. (2009)
Cordata (Amphibia)	*Litoria fallax*	Australia	mtDNA	Φ_{ST}, Excoffier et al. (1992)	22	87	Yes	James and Moritz (2000)
Cordata (Amphibia)	*Rana cascadae*	USA	STR	F_{ST}, Weir and Cockerham (1984)	18	396	Yes	Monsen and Blouin (2004)
Cordata (Amphibia)	*Taricha granulosa*	USA	mtDNA	Φ_{ST}, Excoffier et al. (1992)	19	109	Yes	Kuchta and Tan (2005)
Cordata (Amphibia)	*Taricha granulosa*	USA	Allozyme	Φ_{ST}, Excoffier et al. (1992)	19	109	Yes	Kuchta and Tan (2005)

Table 1 (continued)

Phyla (class)	Species	Area	Genetic information	Statistics	Number of populations	Number of individuals	IBD significance	References
Cordata (Amphibia)	*Taricha torosa sierrae*	USA	mtDNA	Nei ([1978](#)) unbiaised distance	15	77	Yes	Kuchta and Tan ([2006](#))
Cordata (Amphibia)	*Taricha torosa torosa*	USA	mtDNA	Nei ([1978](#)) unbiaised distance	8	32	Yes	Kuchta and Tan ([2006](#))
Cordata (Aves)	*Grus canadensis*	North America	STR	F_{ST}, Weir and Cockerham ([1984](#))	3	191	Yes	Jones et al. ([2005](#))
Cordata (Aves)	*Strix aluco*	Europe	mtDNA	N_{ST}, Pons and Petit ([1996](#))	14	184	No	Brito ([2007](#))
Cordata (Aves)	*Strix aluco*	Europe	STR	R_{ST}, Slatkin ([1995](#))	14	184	No	Brito ([2007](#))
Cordata (Aves)	*Tympanuchus cupido*	USA	mtDNA	Φ_{ST}, Excoffier et al. ([1992](#))	5	341	Yes	Johnson et al. ([2003](#))
Cordata (Aves)	*Tympanuchus cupido*	USA	STR	F_{ST}, Weir and Cockerham ([1984](#))	5	341	No	Johnson et al. ([2003](#))
Cordata (Mammalia)	*Meles meles*	Europe	STR	F_{ST}, Weir and Cockerham ([1984](#))	21	519	Yes	Pope et al. ([2006](#))

Phyla (Class) and Species provide the taxonomic information. *eusocial species. Area provides the geographic area considered for IBD assessment. Genetic information displays the kind of genetic data used for IBD assessment (*mtDNA* mitochondrial gene sequence, *rDNA* ribosomal gene sequence, *STR* short tandem repeats; or Allozyme). Statistics show the statistic method used for the IBD assessment. IBD shows if a significant IBD pattern has been detected (yes/no). References provide the reference of the study where the IBD assessment was performed

loci. Indeed, the number of species with significant IBD patterns are quite similar between the two types of loci. However, slightly more significant IBD signals were identified in mitochondrial than in nuclear datasets (Table 2).

Although long-distance migrations of bee females are known (e.g. Vepsalainen and Savolainen 2000), many bee males have higher flight distance capacity than females (e.g. Kraus et al. 2009; Lepais et al. 2010). This difference can further be increased by the female philopatry often observed in bee species (e.g. Yanega 1990; Cerântola et al. 2011; López-Uribe et al. 2015) although all bee females are not philopatric (e.g. *B. terrestris*, Erler and Lattorff 2010). Therefore, a sex-based effect can be expected with more significant IBD signals identified for maternal mitochondrial DNA-based datasets. This could explain the difference of IBD patterns between our analyses based on mitochondrial or nuclear loci. Further analyses of potential sex-biased dispersal (e.g. Prugnolle and de Meeus 2002) is needed to assess this hypothesis. Alternatively, the difference between mitochondrial and nuclear loci could be likely related to the lower population effective size associated with mitochondrial genes (Avise 2000). Indeed, this leads to higher genetic drifts and phylogeographic signal for mitochondrial genes. Nevertheless, the expected differences due to different effective sizes between mitochondrial and nuclear loci are less important in haplo-diploid

organisms such as bees (Crozier 1977) compared to diploid species.

Combining mitochondrial and nuclear markers in inter-individual distance approach results in similar IBD patterns as those based on COI (Table 2). This is most likely due to the stronger phylogeographic signal for this mitochondrial fragment compared to nuclear loci (Lecocq et al. 2013a; Dellicour et al. 2016). In contrast, combination in inter-population distance approach leads to the detection of less significant IBD signals among studied species compared to loci independently analyzed. We hypothesize that this result is a consequence of (i) an overall weaker IBD signal in at least one of the two kinds of loci or (ii) an IBD signal supported by different pairs of populations within the mitochondrial and nuclear datasets. In both cases, this would decrease the IBD signal estimated from the combined distance statistics.

Limitations of the IBD approaches

The accuracy of the inter-specific comparison of IBD patterns relies on the genetic datasets used and the IBD assessment methods.

The use of different genetic markers for each species to assess IBD patterns could be problematic for inter-specific comparisons. For instance, a comparison of IBD patterns

Table 2 Isolation by distance between population pairs based on different inter-populations and inter-individuals genetic distances in nine European bee species

	Inter-populations distance $\Phi_{ST}/(1-\Phi_{ST})$				Inter-individuals distance IID			
	LR R^2	LR R^2 p value	Mantel r statistic	Mantel p value	LR R^2	LR R^2 p value	Mantel r statistic	Mantel p value
Mitochondrial locus								
Bombus gerstaeckeri	0.056	0.101	0.237	0.132	0.041	**<0.001**	0.204	**0.001**
Bombus hortorum	0.271	**<0.001**	0.520	**0.021**	0.398	**<0.001**	0.631	**<0.001**
Bombus lapidarius	0.016	**<0.001**	0.126	0.100	0.027	**<0.001**	0.163	**<0.001**
Bombus pascuorum	0.001	0.290	0.03	0.337	0.061	**<0.001**	0.247	**<0.001**
Bombus pratorum	0.122	**<0.001**	0.350	**0.003**	0.375	**<0.001**	0.612	**<0.001**
Bombus terrestris	0.003	0.547	0.056	0.345	0.000	0.691	0.005	0.404
Melitta leporina	0.070	**<0.001**	0.265	**0.001**	0.051	**<0.001**	0.227	**<0.001**
Melitta nigricans	0.028	**<0.001**	0.166	**0.047**	0.008	**<0.001**	0.088	0.012
Melitta tricincta	0.000	0.936	0.006	0.392	0.022	**<0.001**	0.148	**0.008**
Nuclear loci								
Bombus gerstaeckeri	0.022	0.357	0.15	0.289	0.212	**<0.001**	0.460	**<0.001**
Bombus hortorum	–	–	–	–	0.002*	0.201*	−0.043	0.621
Bombus lapidarius	0.000	0.633	0.014	0.352	0.021	**<0.001**	0.146	**<0.001**
Bombus pascuorum	0.072	**<0.001**	0.268	**0.025**	0.28	**<0.001**	0.529	**<0.001**
Bombus pratorum	0.012	**0.007**	0.111	0.118	0.005	**<0.001**	0.068	0.108
Bombus terrestris	0.081	**<0.001**	0.285	**0.026**	0.004	**<0.001**	0.063	0.132
Melitta leporina	0.028	**<0.001**	0.168	**0.026**	0.001	0.037	0.025	0.227
Melitta nigricans	0.005	0.076	0.073	0.157	0.003*	<0.001*	−0.054	0.93
Melitta tricincta	0.000*	0.807*	−0.017	0.498	0.035	**<0.001**	0.186	**<0.001**
All loci combined								
Bombus gerstaeckeri	0.035	0.183	0.187	0.121	0.063	**<0.001**	0.250	**<0.001**
Bombus hortorum	0.002	0.641	0.041	0.329	0.326	**<0.001**	0.571	**<0.001**
Bombus lapidarius	0.008*	0.003*	**−0.088**	0.954	0.038	**<0.001**	0.196	**<0.001**
Bombus pascuorum	0.017	**<0.001**	0.132	**0.008**	0.261	**<0.001**	0.511	**<0.001**
Bombus pratorum	0.001*	0.541*	−0.029	0.651	0.302	**<0.001**	0.549	**<0.001**
Bombus terrestris	0.001	0.673	0.034	0.338	0.000	0.339	0.013	0.330
Melitta leporina	0.001*	0.346*	−0.036	0.727	0.007	**<0.001**	0.083	**<0.001**
Melitta nigricans	0.008*	**0.021***	−0.091	0.963	0.001	**<0.001**	0.023	0.246
Melitta tricincta	0.000	0.996	0.000	0.474	0.021	**<0.001**	0.147	**0.001**

"LR" refers to "linear regression". "R^2" refers to the linear regression determination coefficient. *Refers to linear regression associated with a negative coefficient value, and "−" means that there are too few interpopulational Φ_{ST} to compute LR R^2 and Mantel tests. p values smaller than 0.05 and associated with a positive LR coefficient or Mantel r statistic are highlighted in bold

detected through analyses of different genomes (e.g. mitochondrial loci vs. nuclear loci) could make any attempt to generalize the results somewhat doubtful because of the specificities of each genome (e.g. differentiation impacted by sex-specific dispersal; higher or lower mutation rate). Even the use of different loci from the same genome can lead to very different IBD patterns due to the large differences in rates of polymorphism between loci (e.g. Lecocq et al. 2013a). Such an issue could be expected in our analyses as the compared IBD patterns are based on different genetic datasets. However, we speculate that such divergences are here smoothed because our IBD patterns of the nine European bee species are detected by using multiple

markers from different genomes. This provides a more complete and convincing picture of IBD pattern in species less dependent on the choice of genetic markers. The subsequent inter-specific comparison of IBD are thus based on an overview of genetic information minimizing the divergences between loci. Nevertheless, our inter-specific comparison should be considered carefully, especially for genetic datasets of species mined from the literature for which few different loci are available.

Some limitations of IBD signal detection analyses could also result in inaccurate IBD assessments, which would make a comparison of studies less valuable. On one hand, temporal instability of genetic structure (i.e. temporal

Table 3 Isolation by distance between population pairs based on different inter-populations and inter-individuals genetic distances in six non-European bee species

	Inter-populations distance $\Phi_{ST}/(1-\Phi_{ST})$				Inter-individuals distance IID			
	LR R^2	LR R^2 p value	Mantel r statistic	Mantel p value	LR R^2	LR R^2 p value	Mantel r statistic	Mantel p value
Mitochondrial locus								
Bombus ephippiatus	0.035*	0.035*	−0.188	0.853	0.059	<0.001	0.243	**<0.001**
Eulaema bombiformis	0.029*	0.087*	−0.171	0.783	0.398	**<0.001**	0.631	**<0.001**
Eulaema cingulata	0.003	0.481	0.053	0.377	0.089	**<0.001**	0.298	**<0.001**
Eulaema meriana	0.171*	0.000*	−0.414	0.995	0.034	**<0.001**	0.186	**<0.001**
Melipona quadrifasciata	0.000	0.763	0.009	0.462	0.393	**<0.001**	0.627	**<0.001**
Ribosomal locus								
Bombus trifasciatus	0.014*	0.690*	−0.117	0.653	0.113	**<0.001**	0.336	**<0.001**

"LR" refers to "linear regression". "R^2" refers to the linear regression determination coefficient. *refers to linear regression associated with a negative coefficient value, and "−" means that there are too few interpopulational Φ_{ST} to compute LR R^2 and Mantel tests. p values smaller than 0.05 and associated with a positive LR coefficient or Mantel r statistic are highlighted in bold

genetic variation, which can exceed the geographic component) can lead to temporary IBD patterns in particular species which are not persistent through time. This makes the interspecific comparison of IBD patterns doubtful without temporal replication (Dannewitz et al. 2005). On the other hand, the scale of sampling can affect IBD estimation. Indeed, IBD relationships can vary over different spatial scales and geographic regions (Petrou et al. 2014). This makes useless the comparison between species sampled at different scales or between species with inappropriate sampling scales. Overcoming these limitations remains difficult since it requires a fine-scale sampling with temporal replicates across the species range, which is not in easy reach for most species.

Beside this potential sampling bias, the widely used inter-population approach could be suboptimal for IBD assessment. Indeed, our results based on the inter-individual and the inter-population approaches lead to rather different biological conclusions (Table 2). These divergences are most likely a consequence of how individuals are grouped. Φ_{ST}-based IBD estimations imply grouping samples into populations. This assumption could affect the reliability of IBD estimation in four ways. First, the population delimitation is commonly arbitrary defined (e.g. here by sampling localities), which can lead to biologically irrelevant grouping (Storfer et al. 2010; Lotterhos 2012), especially for widespread and ubiquitous species. Alternative more relevant grouping *a priori* could be proposed by taking into account the species ecology. For instance, grouping individuals of a social species (e.g. *Bombus* spp.) collected in the same nest would be a biologically more meaningful approach. However, this requires that field sampling has been designed adequately (e.g. in the present study, specimens have been collected on flowers and not in nests, which prevents a grouping based on nesting sites). Second,

a population-based approach hides intra-population differentiation: individuals from the same arbitrary defined population can be genetically distant and potentially display a significant IBD pattern at a smaller geographic scale, which should be included in the species-range IBD assessment. Third, grouping individuals decreases the number of available pairwise values to estimate such IBD patterns. For instance, in the case of the *B. lapidarius* sampling (236 individuals grouped in 50 user-defined populations/sampling localities), we have potentially 1225 pairwise Φ_{ST} values at the inter-populations level versus 27,730 pairwise IID values at the inter-individual level. This drastically reduces the information available for IBD assessment. Fourth, a small number of individuals per population can make impossible the Φ_{ST} calculation and, similarly, reduces the information available for IBD assessment. Therefore, we speculate that the inter-population approach could, in some situations, result in inaccurate IBD estimations, and should therefore be replaced by the inter-individual approach. While this latter approach remains uncommonly used, it has been previously suggested as an improvement for IBD assessment (Rousset 2000) and landscape genetic studies (Manel et al. 2005; Segelbacher et al. 2010).

IBD patterns in bee species

The divergent results obtained by the inter-individual and the inter-population approaches imply different conclusions on the occurrence of IBD in bee species. While we argue for the use of inter-individual approach (see "Limitations of the IBD approaches" section), we present the conclusions of both approaches since their respective limitations need further investigations. Moreover, results based on the inter-population approach are the only ones available for the data from literature of species non here re-analyzed (Table 1).

The analyses of the nine European and the six other bee species based on the inter-individual approach strongly suggest IBD is a common phenomenon in this species group. Therefore, IBD phenomenon does not seem to be specific to a particular group of bee species but shared by all bee species across the world. Therefore, this general pattern can be explained by intrinsic characteristics of bee that limit their species range-wide gene flow such as (i) philopatry of several species (e.g. Yanega 1990; Cerântola et al. 2011; López-Uribe et al. 2015) and (ii) relatively limited flight distance capacity (e.g. Kraus et al. 2009; Lepais et al. 2010; even long-distance migrations occur between adjacent regions, e.g.; Vepsalainen and Savolainen 2000). In contrast, *B. terrestris* and *M. nigricans* do not display a significant IBD patterns for all loci (Tables 2, 3). This is most likely a consequence of their specific demographic history. First, the recent demographic history of *M. nigricans* (i.e. that has likely experienced a more recent range expansion; Dellicour et al. 2015b) could result in lower of even non-significant IBD signals compared with that of long-term established species ranges. Second, *B. terrestris* is massively produced and traded to improve pollination of many crops since the 1980s (Velthuis and van Doorn 2006). Domestication process associated to development of *B. terrestris* production industry has focused on particular subspecies and differentiated populations due to their intrinsic valuable features for bombiculture (Velthuis and van Doorn 2006; Kwon 2008). Therefore, several *B. terrestris* subspecies have been reared and introduced in areas where other populations or subspecies occurred during the last 20 years (Lecocq et al. 2016a). Such human-mediated translocations could have led to genetic homogenization, erasing of past population structure (Lecocq et al. 2016a) and potential past IBD pattern.

The results of inter-individual approach show that IBD does not occur in all bee species (Tables 2, 3). Similar conclusions are underlined by literature for bee and non-bee species (Table 1). Considering only European animal species, comparison between previous studies does not suggest a common IBD pattern but rather species-specific patterns even within the same organism group (Maes and Volckaert 2002; Schmitt et al. 2003; Brito 2007). Within the Apoidea Anthophila (i.e. bees), a study of six North American bumblebees (Lozier et al. 2011), six non-European bees here re-analyzed, and our population-based results both suggest that IBD is a species-specific feature. Moreover, IBD patterns comparisons between (i) eusocial bees (e.g. *Bombus* spp. and *Melipona quadrifasciata*) and solitary bees (e.g. *Melitta* spp. and *Eulaema* spp.), (ii) generalist pollen foragers (e.g. *Bombus* spp. and *Melipona quadrifasciata*) and specialist pollen foragers (e.g. *Melitta* spp. and *Eulaema* spp.), (iii) large size species (e.g. *Bombus* spp. and *Eulaema* spp.) and medium size species (e.g. *Melitta* spp.) do not show general trends between and within these groups. This could suggest that the IBD phenomenon is not specific to a particular taxonomic group (above the species-level), ecological trait or geographic region. This species-dependent IBD could be a consequence of (i) the species-specific dispersal capacity (dispersal distances can vary between species even within the same genus; e.g. Kraus et al. 2009; Lepais et al. 2010), or (ii) the specific demographic history of a species (Hewitt 1996; Lecocq et al. 2013a; Dellicour et al. 2014, 2016) that can lead to a distribution of genetic differentiation shaped by alternative geographic processes (e.g. a suture zone).

Conservation implications

According to the results based on inter-individual distance, a multi-local conservation approach seems the most appropriate for almost all the wild bee species considered. Indeed, their low but significant IBD patterns indicate a relatively limited species-range connectivity making such species threaten by regional extinction risk and subsequently by strong endangerment (Frankham et al. 2010). Although IBD patterns is mainly shaped by limited species migration capacity due to intrinsic factors or biogeographic barriers over a long time period, recent fragmentation of bee habitats (Winfree 2010) most likely will further decrease the species-range wide connectivity. Such a conclusion should encourage managers to distribute conservation efforts on most distant populations as possible. This would allow ensuring persistence of most species throughout their range and preserving bee species diversity related to the biotic pollination efficiency (Garibaldi et al. 2013) over large geographic areas. Nevertheless, although a multi-local conservation is appropriate for a management approach, preserving large population sizes all along their distribution ranges is essential to maintain durable populations. Since global surface of protected areas are unevenly distributed over large areas (see World Database on Protected Areas), complementary landscape management practices should be used in order to develop an efficient multi-local conservation network across the species ranges. This requires global landscape management based on agro-environmental landscapes and agricultural practices favorable toward pollinators, as well as the integration of urban/semi-natural habitat landscape transitions in conservation planning (Steffan-Dewenter et al. 2002; Perfecto and Vandermeer 2010; Fortel et al. 2014). Alternatively, a decrease of local risk of extinction could be achieved through artificial manipulations of gene flow between distant populations thanks to natural corridors (Holzschuh et al. 2009) or specimen translocations (Gammans et al. 2009; Kerr et al. 2015 but see potential issues in; Lozier et al. 2015).

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