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Molecular taxonomic analysis of the plant associations of adult pollen beetles (Nitidulidae; Meligethinae), and the population structure of *Brassicogethes aeneus*

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Abstract:

Pollen beetles (Nitidulidae, Meligethinae) are among the most abundant flower-visiting insects in Europe. While some species damage millions of hectares of crops annually, the biology of many species is little known. We assessed the utility of a 797 base pair fragment of the cytochrome oxidase 1 gene to resolve Molecular Operational Taxonomic Units in 750 adult pollen beetles sampled from flowers of 63 plant species sampled across the UK and continental Europe. We used the same locus to analyse region-scale patterns in population structure and demography in an economically important pest*, Brassicogethes aeneus.* We identified 44 Meligethinae at *ca.* 2% divergence, 35 of which contained published sequences. A few specimens could not be identified because the MOTUs containing them included published sequences for multiple Linnaean species, suggesting either retention of ancestral haplotype polymorphism or identification errors in published sequences. Over 90% of UK specimens were identifiable as *Brassicogethes aeneus*. Plant associations of adult *B. aeneus* were found to be far wider taxonomically than for their larvae. UK *Brassicogethes aeneus* populations showed contrasting affiliations between the north (most similar to Scandinavia and the Baltic) and south (most similar to western continental Europe), with strong signatures of population growth in the south.

Keywords:

DNA barcodes; *Brassicogethes*; Meligethinae; Pollen beetles; pollinators

Résumé: Les méligèthes (Nitidulidae, Meligethinae), dont certaines espèces endommagent annuellement plusieurs millions d'hectares, sont parmi les insectes floricoles les plus abondants

d’Europe. Cependant leur biologie est pour la plupart largement méconnue. Nous avons évalué la pertinence d'un fragment de 797 paires de bases (pb) du gène codant pour la cytochrome oxydase 1 (CO1), amplifié en utilisant les primers 'Pat' et 'Jerry' de Simons et al. (1994), pour résoudre les MOTUs (Unités Taxonomiques Opérationnelles Moléculaires) chez les Meligethinae; dans un échantillon de 756 spécimens adultes capturés sur 63 espèces végétales de 15 familles différentes échantillonnées en Grande Bretagne et dans 12 pays d'Europe continentale. Nous avons utilisé le même locus pour analyser à une échelle régionale la démographie et la structure de la population d’un ravageur économiquement important : *Brassicogethes aeneus.* Nous avons identifié 44 MOTUs de Meligethinae présentant une divergence de ca. 2% dont 35 contiennent des séquences publiées. Quelques spécimens, contenant des MOTUs incluant des séquences liées à plusieurs espèces linnéennes, n’ont pu être identifiés, ce qui laisse supposer soit une rétention de polymorphismes d’haplotypes ancestraux, soit des erreurs d’identifications dans les séquences publiées. Plus de 90% des spécimens capturés au Royaume-Uni ont été attribués au MOTU correspondant à *Brassicogethes aeneus*. Les associations entre plantes et *B. aeneus* adultes se sont révélées nettement plus diversifiées qu’au stade larvaire. En Grande Bretagne, les populations de *Brassicogethes aeneus* présentent une affiliation différente entre le nord (plus proche des populations scandinaves et baltes) et le sud (plus semblable aux populations d'Europe de l'ouest), avec de forts signes de développement des populations vers le sud.

Mots-clés : Code-barres génétique; *Brassicogethes*; Meligethinae; méligèthes; pollinisateurs

Introduction

Pollen beetles (Meligethinae) are tiny but sometimes superabundant flower visitors across the Holarctic, Afrotropics and Oriental regions (Audisio et al. 2009). Their relative abundance across a range of habitats is shown by the fact that a recent survey found them to comprise over 25% of all flower visitors across UK urban, nature reserve and farmland habitats (Baldock et al. 2015). The two most frequently recorded UK Meligethinae are pest species, *Brassicogethes aeneus* (syn. *Meligethes aeneus* Fab.) and *B. viridescens* (syn. *Meligethes viridescens* Fab.) (Hokkanen 2000; Alford 2003; Olfert and Weiss 2006; Veromann et al. 2006). As adults, both are 2-3mm long, with dark-metallic colouration and superficially similar morphologies. Hibernating adults become active in early spring and attain sexual maturity by feeding on spring-flowering plants in a range of families (Free and Williams 1978). They then migrate to the flower buds of yellow Brassicaceae such as winter oil-seed rape, where they feed and oviposit (Kirk-Spriggs 1996). The larvae feed within the flowers before falling to the ground to pupate (Cook et al. 2004). The new generation of adults emerges in midsummer and feeds on the pollen of a wider range of plant species (Free and Williams 1978), building up the fat reserves required to overwinter successfully. In contrast to work on larval host-plants (e.g. Kirk-Spriggs 1996; Trizzino et al. 2009), the food-plant associations of adult pollen beetles are not widely reported. Adult associations may nevertheless influence population dynamics through impacts on adult maturation, overwinter survival and recruitment to successive generations (Free and Williams 1978; Veromann et al. 2014). Activities of adults and larvae reduce plant fitness both directly (by consumption) and indirectly (through impacts on pollinator visitation rates) (Kirk-Spriggs 1996; Krupnick and Weis 1999). There is evidence that pollen beetles can also act as pollinators; adults in flowers have pollen on their bodies and can disperse pollen at both within-field and landscape scales (Williams *pers. comm.*; Ramsay et al.

2003), and for some plant species they are thought to be the dominant pollinating insect (Gómez 2003; Alonso 2004).

The winged adults of some pollen beetles are able to disperse over large distances with the assistance of prevailing wind currents (Tamir et al. 1967; Chapman et al. 2012). Genetic analyses of European populations also suggest high dispersal, with low differentiation between populations across Sweden (Kazachkova et al. 2007; Kazachkova et al. 2008), and between Lithuania and Finland(Makünas 2012), and more significant (though still low) differentiation between samples from Denmark, France, Finland, Germany, Sweden, and the UK (Kazachkova et al. 2008). The structure of pollen beetle populations is of considerable applied interest because of increasing resistance of pest species to some pesticides (Hansen 2003; Kupfer and Schröder 2015) and possible population variation in the ambient temperatures at which adult dispersal, and hence crop infestation, occurs. Spatial scales of dispersal are also important in predicting range expansion, and at least one species - *B. viridescens* - is introduced and invasive in the Nearctic (Mason et al. 2003; Olfert and Weiss 2006). Understanding of the impacts of these insects, including adaptive responses to pesticides (Zimmer et al. 2014) and environmental change (Hokkanen 2000) requires enhanced understanding of their taxonomy, plant associations, and population structure.

Adult pollen beetles can be identified by specialists using morphological criteria, though identification of larval instars to species is much more difficult (Audisio et al. 2009; Audisio and Jelinek 2015). Kirk-Spriggs (1996) recognised 37 UK species of Meligethinae, and a recent genus-level revision (Audisio et al. 2009) identified ten genera in the UK fauna (*Acanthogethes, Afrogethes*, *Boragogethes*, *Brassicogethes*, *Genistogethes*, *Lamiogethes*, *Sagittogethes*, *Stachygethes*, *Thymogethes*, and *Xerogethes*). A growing body of work has applied molecular

taxonomic approaches to this group (Audisio et al. 2000; Audisio et al. 2002; Trizzino et al. 2009) which, due to the challenges it poses for morphological identification, is eminently suitable for molecular taxonomy. Our study aimed to assess the utility of DNA sequence-based molecular operational taxonomic units (MOTUs) to (i) estimate Meligethinae beetle species richness in a range of UK habitats; (ii) identify adult food-plant associations of pollen beetle MOTUs and relate these to known larval food-plant associations; and (iii) identify Europe-wide geographic and demographic patterns in haplotype distributions for the pest species *Brassicogethes aeneus*.

Materials and methods

Specimen sampling strategy

Sampling for this study comprised 756 new sequences for beetles from 14 European countries (see map, Fig.S1. Locations are also provided as .kmz file suitable for Google Earth in File S1), sampled from 63 plant species in 15 angiosperm families (Table S1, Fig.S2). Our analyses incorporated a further 82 published Meligethinae sequences. Individual level metadata and accession numbers for new and previously published sequences are provided in Table S1.

The sampling for this study was divided into three components.

(i) 365 specimens were drawn from sampling by the UK Urban Pollinators Project (UPP) (Baldock et al. 2015) in 2011 from sites centred on 12 cities spanning the UK, in the southwest (Bristol, Cardiff, Swindon, Southampton), southeast (London, Reading), northeast (Hull, Leeds, Sheffield) and Scotland (Dundee, Edinburgh, Glasgow). Specimens were collected from 39 plant species in 10 families (Fig.S2) during 1 km walked transects in one of three habitat types - nature reserve, farm, and urban - around each city (see Baldock et al. (2015) for full details on site selection and habitat

categories). Our subsampling included 222 specimens from farmland, 132 from nature reserves and 11 from urban sites (Table 1). All host plants from which specimens were collected were identified based on direct observations using Stace (2010). Farmland specimens were most frequently sampled from *Brassica napus* sbsp. *oleifera* (Brassicaceae, 29% of specimens) and *Ranunculus repens* (Ranunculaceae, 21%), while nature reserve specimens were most frequently sampled from *Cirsium arvense* (Asteraceae, 18%) and *Rubus fruticosus* (Rosaceae, 14%) (Table S1). Insect specimens were identified to genus morphologically by taxonomists at the National Museum of Wales, Cardiff, and have been deposited in the specimen archive of the UK Insect Pollinators Initiative (Vanbergen et al. 2014) at the Natural History Museum, London, with NHM accession numbers in Table S1.

(ii) To provide wider phylogeographic perspective we sequenced a further 391 adult specimens from additional sites in the UK and 13 continental European countries (Table 1), ranging from the Outer Hebrides islands in the north west of the UK to Romania in south east Europe. This represents the widest geographic sampling of Meligethinae published to date. To increase the probability of extensive sampling of *Brassicogethes aeneus* for population-level analysis, 60% of the additional specimens were collected from *Brassica napus* sbsp. *oleifera*.

(iii) Our analyses included 82 previously published sequences for specimens from 12 European countries, all of which have Linnaean names but lack associated plant data (Table S1). Published sequences included those for vouchers at the Natural History Museum, London, for the commonest UK species (*B. aeneus* and *B. viridescens*) and sequences for 36 additional Meligethinae species from the genera *Afrogethes* (8 species), *Acanthogethes* (1 species), *Boragogethes* (1 species), *Brassicogethes* (11 species), *Genistogethes* (1 species), *Lamiogethes* (5 species), *Meligethes* (3 species), *Sagittogethes* (3 species), *Stachygethes* (1 species), *Thymogethes*

(1 species) and *Pria dulcamarae*. The published sequences include 19 of the 36 species recorded from the UK (Kirk-Spriggs 1996).

DNA extraction

A single leg of each adult beetle specimen was crushed using forceps to break the exoskeleton. DNA was extracted using a chelex protocol following Nicholls et al. (2010). The leg was incubated overnight at 37˚C in a 1.5mL eppendorf tube containing 50µL 5% chelex resin solution and 5µL of 10mg/mL Proteinase K. After incubation, each sample was mixed, centrifuged, heated for 15 minutes at 95˚C to denature any remaining Prot K, re-centrifuged and then stored at -20˚C prior to use in PCR.

PCR and sequencing

We amplified the 797 base pair (bp) fragment of the cytochrome oxidase 1 gene (CO1) available in Genbank for the widest diversity of Meligethinae species at the start of the project. This fragment was amplified using primers SJerryF and SPatR developed by Timmermans et al. (2010) and modified from C1-J-2183 (Jerry) and TL2-N-3014 (Pat) in Simons et al. (1994). This region has been widely applied in studies of beetle phylogenetics, phylogeography and DNA taxonomy because it is more easily amplified in some taxa and can contain greater phylogenetic signal than the standard Folmer barcode region of the same gene (Cardoso and Vogler 2005; Gómez-Zurita et al. 2010; Kubisz et al. 2012). In pollen beetles we found the LCO/HCO primers failed to produce bands for some specimens at an annealing temperature of 51°C and produced multiple bands when initial PCR cycles used a lower annealing temperature of 45°C (Hebert et al.

2004). The Pat/Jerry region does not overlap with the standard Folmer barcode fragment, for which extensive resources for Meligethinae are now available on the Barcoding of Life BOLDSYSTEMS database (accessed 9 January 2016). The fragment that we used proved informative both in allocating specimens to MOTUs and in resolving the population structure and demographic status of populations

PCRs used the following reaction mix and primers: 12.94µL MilliQ water, 2µL 10mg/ml BSA, 2µL 10×reaction buffer, 1µL 50mM $MgCl_2$, 0.16µL 25mM dNTPs, 0.1µL 5U/µL Taq polymerase, 0.3µL 20µM primer SJerryF (5'CAACATYTATTYTGATTYTTTGG3'), 0.3µL 20µM primer SPatR (5'GCACTAWTCTGCCATATTAGA3') and 1.2µL template DNA. The PCR program used was 94°C for 2 minutes, 35 cycles of (94°C for 30 seconds, 51°C for 30 seconds, 72°C for 1 minute), 72°C for 5 minutes, then hold at 10°C. PCR success was checked by running 3µl on a 2% agarose gel, and the remainder of each reaction was prepared for sequencing by adding 2.5µL of a 0.4U/µL Shrimp Alkaline Phosphatase and 0.6U/µL Exonuclease 1 (SAP/EXO 1) mix to each PCR reaction (incubating for 37°C for 40 minutes and 94°C for 15 minutes) to remove unincorporated dNTPs and primers. Samples were sequenced using ABI BigDye Terminator version 3.1 sequencing chemistry (Applied Biosystems) and run on an ABI 3730 capillary machine by the Edinburgh Genomics NERC facility.

Sequence alignment and phylogenetic analysis

Sequences were edited and checked for an appropriate open reading frame (to eliminate possible nuclear pseudogenes - NUMTs; Bensasson et al. 2001) using Sequencher version 5.01 (Gene Codes Corporation, Ann Arbor MI, USA) and aligned using the Clustal W algorithm in

MegAlign v5.05 (DNAstar Inc., Madison WI, USA). After editing, all CO1 sequences were 797bp long, and the completed alignment was checked by eye. Sequences and Genbank Accession numbers (**to be added on acceptance**) for each accession are given in Table S1. For inference of phylogenetic relationships we generated a trimmed alignment in which duplicate haplotypes from the same sampling location were removed using Collapse v.1.2 (Posada 2013), leaving 241 haplotypes including the outgroup *Kateretes rufilabris* from the family Kateretidae, sister taxon to the Nitidulidae (Genbank accession number DQ221966; Cline et al. 2014). An appropriate model of sequence evolution for our data was identified using MrModeltest v2.3 (Nylander 2004) as GTR+I+G. This model was used in Bayesian inference of phylogenetic relationships in the software MrBayes 3 (Ronquist and Huelsenbeck 2003). The MrBayes analysis ran for 2.5 million iterations, with 1 cold chain and 3 heated chains using default heat parameters, after which the average standard deviation of split frequencies was 0.02. We used a burn-in of 250,000 generations and checked parameter posterior distributions for convergence in Geneious. No molecular clock was enforced.

Molecular taxonomic analysis

Similarity of new data to published sequences was examined in the first instance using nucleotide BLAST search (Altschul et al. 1990). Sequences from samples identified through BLAST as Meligethinae or its outgroup *Kateretes rufilabris* (788 newly generated and published sequences) were allocated to molecular operational taxonomic units (MOTUs) using two approaches: jMOTU v1.0.8 (Jones et al. 2011) and ABGD (downloaded July 2014) (Puillandre et al. 2012). jMOTU clusters sequences into MOTUs that differ by pre-defined numbers of bases; we examined divergence distances amongst sequences ranging from 1-80 bp, with a low BLAST

identity filter of 97%. In the presence of a barcoding gap, the plot of MOTU by divergence should form a plateau, with no change in MOTU number across the divergence levels corresponding to the gap.

ABGD defines MOTUs based upon prior values of within-species divergence, and assesses how MOTU number changes as within-species divergence increases. We used prior within-species divergence limits ranging from 0.4% to 10%, split into 30 steps; K2P distances were used, with a Ti/Tv ratio of 1.45 (calculated by MrModeltest), and using the default value of 1.5 for slope increase. Output from the recursive partitioning scheme was used, with the final number of MOTUs chosen at the point where the plot of MOTU versus intraspecific divergence levelled off.

Analysis of population genetic structure and demography

We analysed population genetic differentiation and demography only for the single most abundant MOTU, corresponding to *Brassicogethes aeneus* (n=635), using the package Arlequin (Excoffier et al. 2005). Our aim was to understand the spatial scale of haplotype variation in the UK, and to place UK variation in a broader European context. We used analyses of molecular variance (AMOVA) to quantify population genetic structure at three nested spatial scales (specified fully in Table S2a):

(a) Between locations within each region of the UK.
(b) Between 4 regions of the UK (Scotland, NE England, SW England and Wales, and SE England), and
(c) Between five regions of Europe (Northern UK, Southern UK, France/Belgium/Germany, Scandinavia and the Baltic, and Southern Europe - shown in Fig.2);

Our division of the UK into regions in (b) was intended to explore the possibility of latitudinal genetic structure associated with restricted gene flow along relatively narrow habitat corridors of a key foodplant, *Brassica napus* sbsp. *oleifera* agriculture in northern Britain (Botanical Society of Britain and Ireland distribution map, http://bsbidb.org.uk/maps/?taxonid=2cd4p9h.ydh, accessed 19 January 2016). Division of the UK into North and South at the largest spatial scale reflects the results of analyses at the UK level. Our division of continental Europe into three regions *a priori* reflects previous work showing insect dispersal to the UK from the southeast (region France+Belgium+Germany) and from the northeast (region Scandinavia+the Baltic, which includes samples from Sweden, Estonia and Poland) (Williams 1951; Chapman et al. 2002; Brattström et al. 2010; Chapman et al. 2012; Raymond et al. 2013; Stefanescu et al. 2013). Samples from the final region (region Southern Europe, which includes samples from Italy, Austria, Hungary, Romania, Bulgaria and Greece) were included to provide a preliminary assessment of haplotype variation for a region known to support high diversity in many widespread European taxa (e.g. Taberlet et al. 1998; Hewitt 2000; Stone et al. 2012). We were unable to obtain any samples from the Iberian peninsular, though this region often harbours distinct genetic variation in widely distributed taxa and should be included for a comprehensive understanding of Europe-wide patterns (Taberlet et al. 1998; Hewitt 2000). Though patterns at any single locus must be analysed with care (Hurst and Jiggins 2005), patterns in mitochondrial haplotypes remain informative of genetic relationships between populations (e.g. Bradman et al. 2011; Stone et al. 2012; Winkelmann et al. 2013).

We also used AMOVA to test for food-plant family- associated population structure in the same European *Brassicogethes aeneus* MOTU. Because *B. aeneus* larvae are thought to only develop on a Brassicaceae subset of the food-plants visited by adults, and mating occurs in the spring when adults recruit to Brassicaceae after hibernation, our expectation was for there to be

no intraspecific population structure based on adult food-plants. This analysis included Europe-wide sampling of *B. aeneus* from adult food-plants in the families Alliaceae, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Ranunculaceae and Rosaceae. All AMOVAs used 10000 permutations, with 1000 permutations for significance testing of pairwise F_{ST}.

Haplotype diversity in *Brassicogethes aeneus* was illustrated using a minimum spanning network (Bandelt et al. 1999) constructed in the package PopART (http://popart.otago.ac.nz). Pairwise differentiation between sites or groups was quantified using F_{ST} and tested using exact tests in Arlequin (Raymond and Rousset 1995).

The demographic history of *B. aeneus* population units was assessed using haplotype pairwise mismatch distributions and tests of selective neutrality in Arlequin. Mismatch distribution patterns were compared for goodness-of-fit to a model of sudden population expansion using the sum of squared deviations test (Schneider and Excoffier 1999). Departures from selective neutrality indicative of selection or population size change were tested using Tajima's D (Tajima 1989a; Tajima 1989b) and Fu's FS (Fu 1997).

Results

Sequence diversity and phylogenetic relationships between Meligethinae CO1 haplotypes

Across all accessions in our analysis the 797 bp CO1 fragment showed 587 variable sites, with no evidence of nuclear pseudogenes (NUMTs). The amplified CO1 fragment showed low phylogenetic resolution at the generic level, and published sequences for the genera *Afrogethes, Lamiogethes*, and *Sagittogethes* were non-monophyletic in our Bayesian phylogenetic reconstruction (Fig.1, Fig. S3). Sequences for most of the newly-sampled specimens fell into

strongly-supported clades (posterior probability = 1) containing published sequences for one of *Brassicogethes aeneus* or *B. viridescens* (Fig.S3).

Resolution of Meligethinae into Molecular Operational Taxonomic Units.

721 of 756 specimens initially identified as pollen beetles (337/365 UK UPP specimens and 384/391 wider European samples) showed ≥ 98% BLAST sequence similarity to published sequences for Meligethinae. The UPP exceptions included sequences with ≥ 98% match to published data for other small and superficially similar beetles that are frequently found in flowers, including *Hydrothassa marginella* (11 sequences, Chysomelidae)*, Anaspis frontalis* (five sequences, Scraptiidae), *Eusphalerum sorbi* (four sequences, Staphylinidae) *and Epuraea melina* (two sequences, Nitidulidae, Carpophilinae). All non-Meligethinae sequences so identified were excluded from further analyses.

jMOTU analysis of the resulting putative Meligethinae sequences and 81 published Meligethinae sequences (n=788) revealed putative barcoding gaps (Fig.S4) at 1.0-1.4% divergence (8-11 base pairs, n=50 MOTUs) and at 2.0-2.3% divergence (16-18 base pairs, n=44 MOTUs). ABGD gave strong support for 44 MOTUs at 0.78 to 1.7% divergence. Membership of the 44 MOTUs identified by jMOTU and ABGD was almost identical, with only a single individual of the 788 (a Genbank sequence for M. aeneus from Greece, AM491335) changing MOTU membership between the two analyses (shown for all sequences in Table S1). In subsequent analyses we have used the n=44 ABGD MOTU allocations. Phylogenetic relationships between the 44 MOTUs, and the published voucher sequences they contain, are shown in Fig.1 and Fig.S3.

Thirty-five of the MOTUs contain previously published Genbank sequences, leaving nine unidentified. MOTUs at this level show some disagreement with morphology-based allocations to Linnaean species. In four cases, published sequences attributed to a single morphological species were split between two MOTUs: *Brassicogethes viridescens* (MOTUs 13, 44), *B. coracinus* (MOTUs 15, 17), *B. erysimicola* (MOTUs 16, 17) and *Afrogethes fruticola* (MOTUs 26, 27). In contrast, three MOTUs each incorporated published sequences attributed to more than one recognised genus and/or species. This was most dramatic in the case of the eight Linnaean species included in MOTU 17 (*Brassicogethes coracinus, B. arankae, B. erysimicola, B. matronalis, B.* nr *coracinus, B. M2 nr longulus, B. thalassophilus* and *B. longulus*), but was true also for MOTU 8 (2 species: *Lamiogethes bidens, Sagittogethes ovatus*) and MOTU 24 (2 species: *Afrogethes canariensis, Afrogethes isoplexidis*).

DNA sequence-based identification of specimens

ABGD matched 97.6% (all but 17) of putative Meligethinae specimens to MOTUs containing published Meligethinae sequences (Table S1, Fig.1). Ninety-seven percent of UK sampled specimens (326/337 UPP and 51/52 additional non-UPP) were allocated to the single MOTU (30) containing all published sequences for *Brassicogethes aeneus*. The remainder were matched with *Kateretes rufilabris* (MOTU 2, one specimen from Dundee's nature reserve site), *Brassicogethes viridescens* (MOTU 44, n=7: one from Edinburgh's nature reserve site, four from Dundee's farm, one from Glasgow's nature reserve, and one from London's nature reserve), and *Fabogethes nigrescens* (MOTU 36, one from London's farm). Only one UPP specimen, from the Bristol farmland habitat, was allocated to a MOTU (9) lacking any identified reference sequence. In the phylogenetic tree of haplotype sequences (Fig.1 and Fig.S3) this MOTU is placed between MOTU

40 *Stachygethes ruficornis* and MOTU 34, which includes an unidentified pollen beetle from Croatia (see below); without denser taxon sampling and/or use of an additional sequence marker we cannot place this specimen by barcode identification even to genus.

The 339 Meligethinae specimens from non-UPP sites in the UK and continental Europe were allocated to 15 MOTUs; 322 specimens were allocated to eight MOTUs containing previously identified specimens, while 17 specimens (from Italy, France, Croatia and Poland) were allocated to seven MOTUs lacking a published reference sequence (Table S1). Again, the vast majority (91%) of specimens were sequence-matched to *B. aeneus* (MOTU 30, n=309). Smaller numbers of specimens were sequence-matched to *Sagittogethes obscurus* (MOTU 11, n=2, from France), *Brassicogethes viridescens* (MOTU 13, n=17, from Austria and the UK; and MOTU 44, n=28, from the UK, Italy, Sweden, Estonia), *Lamiogethes pedicularius* (MOTU 31, n=8 from Austria) and *Thymogethes gagathinus* (n=2, from Croatia). All of these identifications are consistent with known geographic ranges (Audisio et al. 2009). Six specimens were allocated to MOTUs containing reference sequences for more than one species, preventing unambiguous identification. Three specimens (from Estonia, Bulgaria and Poland) were allocated to multispecies MOTU 17 (the *Brassicogethes coracinus* group in Fig.1, which contains *Brassicogethes coracinus/ B. arankae/ B. erysimicola/ B. matronalis/ B. nr longulus*), and two specimens from Hungary were allocated to MOTU 8 (which contains *Lamiogethes bidens/Sagittogethes ovatus*).

Adult food-plant associations

Adults sequence-matched with *B. aeneus* were sampled from 41 plant species in nine families (Table 2, Fig.S2, Table S1). Only one specimen was sampled from a monocot flower -

Gagea lutea (Liliaceae) in the Bükk Mountains, Hungary. Specimens identified as *B. aeneus* made up 95% of the 305 Meligethinae specimens sampled from *Brassica napus* sbsp. *oleifera* Europe-wide, the other species being *B. viridescens* (4%)*, B. coracinus* and *Fabogethes nigrescens* (<1% each). The dominant flower assocations recorded for *B. aeneus* other than *Brassica napus* sbsp. *oleifera* (44% of specimens) were *Ranunculus repens* (8.3%), *Rubus fruticosus* (6.7%) and *Cirsium arvense* (5%) (Table 2). The flower associations we found for *B. aeneus* match very closely those recorded by Free and Williams (Free and Williams 1978) (Table 2), who also recorded this species from *Arctium vulgare* and *Matricaria matricarioides* (Asteraceae), *Stellaria holostea* (Caryophyllaceae), *Papaver rhoeas* (Papaveraceae), *Prunus avium* (Rosaceae) and *Galium verum* (Rubiaceae). AMOVA showed no evidence of plant family-associated structuring in mitochondrial haplotypes in *B. aeneus*, with less than 1% of variation explained by differences between plant families (Table S2d). However, the flower associations of *B. aeneus* are non-random. If we compare the flower associations of this species at the plant family level with the full set of insect-flower associations for the same sites, using only the Urban Pollinators project data (n = 10477 insect-flower association records), we find that adult *B. aeneus* show a significant preference for Brassicaceae and are less common than expected on flowers of Asteraceae ($\chi^2 = 20.13$, df = 6, p = < 0.001).

Adults of the second most abundant species overall, *Brassicogethes viridescens* (n=43 Europe-wide), were sampled from 17 plant species in 10 families: Asteraceae (*Angelica sylvestris, Brachyglottis* sp.*, Calendula arvensis, Centaurea* sp.*, Cirsium arvense, Cirsium vulgare, Crepis* sp.*, Hieracium* sp.*, Leucanthemum vulgare, Taraxacum* agg.), Boraginaceae (*Symphytum* spp.), Brassicaceae (*Brassica napus*), Campanulaceae (*Campanula* sp.), Fabaceae (*Melilotus albus*), Hypericaceae (*Hypericum* sp.), Oleaceae (*Jasminum* sp.), Onagraceae (*Chamerion angustifolium*),

Ranunculaceae (*Ranunculus arvensis*) and Rosaceae (*Filipendula ulmaria*). In addition to *Brassica napus* sbsp. *oleifera*, *Brassicogethes coracinus* was sampled from *Sinapis alba* (Brassicaceae) and *Fabogethes nigrescens* was sampled from *Crepis* sp. (Asteraceae). *Lamiogethes pedicularius* was sampled from four species of Asteraceae (*Taraxacum* agg., *Arnica montana*, *Hieracium* sp., *Leucanthemum vulgare*) and one of Ranunculaceae (*Ranunculus arvensis*). *Sagittogethes obscurus* was sampled from *Hypochaeris radicata* (Asteraceae); *Thymogethes gagathinus* was sampled from *Potentilla reptans* (Rosaceae).

Population structure and demography of *Brassicogethes aeneus*

Across the UK and continental Europe 634 specimens were sequence-matched to *Brassicogethes aeneus*, distributed across countries and regions of Europe as shown in Fig.2. The *B. aeneus* MOTU contained 120 CO1 haplotypes. The haplotype frequency distribution was very skewed towards rare haplotypes, with 89 haplotypes represented by a single individual, 187 individuals sharing the commonest haplotype, and 402 individuals (>63%) having one of the top three most abundant haplotypes. The haplotype network for *B. aeneus* is shown in Fig.2.

(a) Spatial patterns in population structure

As expected from the overall haplotype distribution the commonest alleles were shared by most sites, such that only a small component of haplotype variation was explained by differences between population units at any spatial scale. At the level of individual UK populations, the only significant genetic differences (exact tests in Arlequin, $p<0.05$) were between Kildonan (on South Uist in the Outer Hebrides Islands of Scotland) and all other UK sites, and between Edinburgh (Scotland) and each of London and Hull (SE and NE England, respectively). When UK sites were

grouped into four regions (Table S2, Scotland, NE England, SE England, and SW England/Wales), differences between regions explained a low (2.5%) but significant ($p<0.01$) component of haplotype variation (AMOVA, Table S2b), with pairwise F_{ST} values ranging from 0.003 between NE and SE England to 0.053 between Scotland and SE England. Only the differences between Scotland and each of NE and SE regions of England were significant (Arlequin, exact tests, $p<0.05$). Genetic differentiation between European regions explained a slightly greater (4.4%) and more significant ($p<0.001$) component of haplotype variation in *B. aeneus* (AMOVA, Table S2c). Pairwise F_{ST} values ranged from 0.014 between Scandinavia+the Baltic and Scotland to 0.096 between the Scandinavia+the Baltic and France+Belgium+Germany, with all pairwise differences significant except that between Scotland and the Baltic region.

(b) Population demography and tests of selective neutrality

Pairwise mismatch distributions were unimodal and compatible with a rapid population expansion model for all regions of Europe except the Baltic, for which rapid population expansion was rejected ($p<0.001$) (Fig.3, Table S2c). In the absence of significant genetic differentiation between Scotland and Scandinavia+the Baltic, a combined dataset also rejected a rapid population expansion model (Fig.3). All five regional groupings showed significantly negative values of Fu's FS, with significantly negative values of Tajima's D for three regions (England/Wales, France+Belgium+Germany, and Southern Europe).

Discussion

Sequence-based identification of pollen beetles

The region of cytochrome oxidase c used in our analysis contains sufficient variation to separate specimens effectively into molecular operational taxonomic units. Identification of 99% of individuals in our samples to 35 reference taxa, in almost all cases to MOTUs containing published sequences for a single Linnaean species, compares favourably with documented DNA-barcoding of other groups (e.g. Ward et al. 2005; Hajibabaei et al. 2006). However, matching to a single species was not possible for the three MOTUs that each contained published sequences for more than one Linnaean species - eight species in the case of MOTU 17. Sharing of mitochondrial haplotypes among species is widely reported, particularly through sharing of ancestral polymorphism or hybridisation in recent radiations of species (e.g. Funk and Omland 2003; Nicholls et al. 2012), and incomplete sorting of ancestral polymorphism has been hypothesised to explain low phylogenetic signal of cytochrome oxidase sequences in pollen beetles (De Biase et al. 2012). Placement of published sequences for representatives of two genera in a single MOTU (MOTU 8, *Lamiogethes bidens* and *Sagittogethes ovatus*) nevertheless suggests possible misidentification of some reference specimens. Future sequence-based identification of Meligethinae should be developed around the standard Folmer barcode fragment of cytochrome oxidase c, for which a growing resource (570 specimen records, including 389 barcodes of 53 species) now exists on the Barcode of Life BOLDSYSTEMS database (accessed 9 January 2016).

The generally low phylogenetic resolution seen at the generic level in our analysis is concordant with other analyses of mtDNA in pollen beetles (Audisio et al. 2009). The tightly-clustered '*B. coracinus* group' (MOTU 17 in Fig.1, with individual sequences shown in Fig.S3) mirrors recent taxonomic work (Audisio et al. 2011; De Biase et al. 2012) suggesting a clade of recently radiated taxa with challenging taxonomy: our sampling fails to resolve the complexes (e.g.

'*subaeneus*', '*coracinus*', '*longulus*') described therein, though our taxon sampling is far from complete.

Species richness and plant associations

Our sampling of 756 specimens was dominated by a single species: the economically important pest *Brassicogethes aeneus*. This comprised 97% of UK specimens, with no evidence of variation in Meligethinae faunas between UK farm and nature reserve habitats. A striking feature of our sampling is that despite being specialist feeders on particular plant families as larvae, the adults were sampled from a wide range of different plant taxa. For example, while *Brassicogethes* species are specialist feeders on Brassicaceae as larvae, the adults of both *Brassicogethes aeneus* and *B. viridescens* were recorded from flowers of nine and 10 families, respectively. Similarly, *Fabogethes nigrescens* (which feeds on Fabaceae as a larva; (Audisio et al. 2009)) and *Lamiogethes pedicularius*, *Sagittogethes obscurus* and *Thymogethes gagathinus* (specialists of Lamiaceae, (Audisio et al. 2009)) were sampled from non-larval food-plants in Asteraceae, Brassicaceae, Ranunculaceae and Rosaceae.

We did not determine whether the adult beetles we collected were feeding on the sampled flowers. We suggest that this is likely, because the primary role of flower associations in these beetles is to provide pollen food for early summer maturation of eggs in the parental generation, and for laying down of overwintering fat reserves in their adult offspring (Free and Williams 1978; Vinatier et al. 2012; Veromann et al. 2014). Once mating is completed in late spring, there is no other reason to be in flowers. Nevertheless, this aspect of adult biology merits further study, for example through quantitative plant DNA barcoding of gut contents against a

panel of plants from which adults have been collected. These methods have been used succeefully to resolve trophic relationships in other beetle taxa (Jurado-Rivera et al. 2009; Navarro et al. 2010; García-Robledo et al. 2013; Kishimoto-Yamada et al. 2013; Kitson et al. 2013; Kajtoch et al. 2015).

The wider adult host-feeding range of some Meligethinae raises the twin questions of the function of adult feeding and the determinants of larval host specificity. If adult feeding is a significant predictor of successful overwintering and maturation to breed in the following year, then understanding the range and relative rates of exploitation of adult food-plants may be important in the population dynamics of otherwise specialist pest species, such as *B. aeneus* (Williams and Free 1978)(Williams and Free 1978). Contrasts in the host-plant range of adults and larvae are the results of adult preference for feeding and oviposition respectively (Cook et al. 2002; Jönsson et al. 2007; Hervé et al. 2014; Kaasik et al. 2014). There is evidence that adult oviposition choices influence the developmental success of larval pollen beetles (Veromann et al. 2014), but little is known about the consequences of plant choice for adult feeding. Our results are compatible with lower constraint on adult food-plant choice. One testable hypothesis is that the larvae, though able to move between flowers on a single plant (Williams and Free 1978), are constrained to acquire the resources they need to reach adulthood within a narrow window of opportunity (Cook et al. 2004; Beduschi et al. 2015). This in turn could have driven the evolution of larval physiological traits matched to the detoxification and assimilation challenges of specific food-plants, resulting in high larval host-plant specificity. In contrast the more mobile adults are able to move between food resources, escaping time constraints on food assimilation efficiency in favour of physiological traits allowing exploitation (perhaps at lower efficiency) of a wider host-plant range over a longer period.

An alternative hypothesis to higher larval than adult food-plant specificity is that the contrasting host ranges of larval and adult stages merely reflect seasonal changes in the availability of highly rewarding pollen sources. When adults emerge from hibernation in April/May, they first feed on early flowering species such as *Salix* spp. and *Anemona nemorosa* (Thieme, personal observation). Later in the spring there are relatively few alternative forb species to cultivated *Brassica napus* sbsp. *oleifera* that are both at high floral density and provide a high pollen volume per flower (see per-species values in Hicks et al. (2016, in press)). This hypothesis is supported by the fact that the non-Brassicaceae host-plants selected by the other spring adult *B. aeneus* in our dataset also provide high pollen volumes per capitulum (*Ficaria verna, Taraxacum* agg.) and/or provide high floral density (*Allium ursinum*). Food-plant associations of newly emerged adults in the summer are compatible with the same hypothesised preference for plants that provide high, spatially-concentrated, pollen resources (e.g. *Cirsium* spp., *Rubus* spp., *Ranunculus* spp., *Taraxacum* agg., *Filipendula ulmaria, Leucanthemum vulgare*) – as are additional food plant associations for pre-winter adults outwith this study (e.g. *Sambucus nigra* and *Tilia* spp., ornamentals such as lilies and roses, and flowers of vegetables such as cauliflower and broccoli; Thieme, personal observation). Further sampling of pollen beetle-plant associations is required to better understand the basis of adult food plant preferences given availability. Given that pollen beetles are often very abundant, their flower associations may be important and information-rich.

These hypotheses could be tested by examining the relative impacts of alternative host-plant selection on larval and adult life stages, with the prediction of greater impacts of host variation on larval rather adult components of fitness. At an applied level, there may be important correlations between damage associated with *B. aeneus* infestation of oil-seed rape crops in early summer and the local or regional abundance of alternative adult food-plants. One possibility is

that such alternative food sources facilitate the build-up of *B.aeneus*, leading to a positive correlation with economic damage (see Free and Williams (1978) on the importance of *Taraxacum* agg. in this regard). An alternative is that high abundance of alternative food sources could reduce beetle abundance on oil-seed rape plants at the crucial green and yellow bud stages, leading to a negative correlation with economic damage. Though there has been extensive study of the impact of landscape characteristics on pollen beetle abundance (e.g. Valantin-Morison et al. 2007; Zaller et al. 2008; Rusch et al. 2012; Beduschi et al. 2015), we know of no studies specifically incorporating the available richness and abundance of adult food-plants.

Population structure and demography of *Brassicogethes aeneus*

The patterns of mitochondrial haplotype differentiation in *B. aeneus* match previous work showing low local differentiation and slightly greater divergence at larger spatial scales (Kazachkova et al. 2007; Kazachkova et al. 2008; Makünas 2012). Our results are novel in showing north-south differentiation at UK and European scales, and low genetic differentiation between Scotland and the Baltic. A selectively neutral interpretation of these patterns is that dispersal in *B. aeneus* is, or has been, primarily longitudinal rather than latitudinal. The patterns in *B. aeneus* contrast with the lack of north-south genetic differentiation seen in species known to undertake latitudinal migrations in Europe, such as the hoverfly *Episyrphus balteatus* (Raymond et al. 2013). Genetic differentiation in *B. aeneus* is nevertheless low (F_{ST} < 0.1 in all regional comparisons), and similar in magnitude to *Episyrphus balteatus* (<0.05, Raymond et al. 2013) and the grain aphid *Sitobion avenae* (<<0.05 Llewellyn et al. 2003).

Without more in-depth analysis using a larger number of markers it is not clear whether the patterns observed in *B. aeneus* represent ongoing gene flow between regional populations, or slow sorting of high levels of ancestral polymorphism in large populations without gene flow. Comparison with patterns in nuclear markers is also required to test the possibility that selection may be influencing mitochondrial haplotype frequencies - either directly, via mito-nuclear interactions, or via co-inherited symbionts such as *Wolbachia* (Hurst and Jiggins 2005; Grant et al. 2006). It is possible that UK north-south differentiation is associated with relatively narrow habitat corridors of *B. napus* sbsp. *oleifera* agriculture in northern Britain (Botanical Society of Britain and Ireland distribution map, http://bsbidb.org.uk/maps/?taxonid=2cd4p9h.ydh, accessed 19 January 2016), restricting adult dispersal and associated gene flow. Similarly, lack of east-west differentiation in the north could be due to occasional large-scale longitudinal migrations, as have been observed for Diamondback moths, *Plutella xylostella* (Chapman et al. 2002; Chapman et al. 2004; Chapman et al. 2012). These reach the UK on warm winds from the east at a similar time of year as pollen beetles, with particularly notable migrations from Scandinavia in the 1960s.

The unimodal mismatch distributions shown by all regional European population of *B. aeneus* except the Baltic are compatible with either rapid population expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992) or range expansion accompanied by high dispersal between populations (Ray et al. 2003; Excoffier 2004). These interpretations are also compatible with the observed negative values of Fu's FS (and in some cases Tajima's D), though these can also indicate purifying selection. The hypothesis of range expansion with high dispersal is further supported by the low absolute levels of genetic divergence observed between regional populations. This interpretation, if correct, suggests that other genetic processes in *B. aeneus,* such as selection for pesticide resistance (Zimmer et al. 2014) may operate on an Europe-wide

spatial scale. Further work using multiple nuclear markers is required to separate the effects of selection from neutral processes, and to discriminate population divergence from subsequent gene flow in *B. aeneus*.

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Figure legends.

Figure 1. Phylogenetic relationships between Meligethinae MOTUs (Molecular Operational Taxonomic Units) (n=44) supported by ABGD. The tree shown is a Bayesian majority rule consensus tree inferred using MrBayes (Ronquist and Huelsenbeck 2003) using a GTR+I+G model of sequence evolution selected using MrModeltest (Nylander 2004), and rooted with a published sequence for *Kateretes rufilabris* (Genbank accession DQ221966). Numbers at nodes indicate posterior probability support. The most species-rich genera in the tree are colour coded as shown in the key. Triangles at branch tips indicate multiple member sequences in a MOTU. Relationships between the full set of 241 unique CO1 haplotypes are shown in Figure S3.

Figure 2. Minimum spanning haplotype network for specimens identified as *Brassicogethes aeneus* (MOTU 30). The 120 sampled haplotypes are shown as filled circles joined by links in the network, while unsampled haplotypes are shown by short transverse lines. Colours in circles show the proportions of samples for a given haplotype sampled from each of the European regional groupings used in AMOVA analyses. Numbers in the inset map show sample sizes by country and (in boxes) by region.

Figure 3. Observed pairwise mismatch distributions for CO1 haplotype sequences from four regional groupings of populations across Europe (in blue) shown alongside the distributions predicted under a model of rapid population growth (in red). Populations in Scotland and the region (Scandinavia + the Baltic) have been pooled to reflect the lack of significant genetic differentiation between them. Analytical summaries for these distributions are provided in Table S2.

Table 1. Summary of sampling for the study. Sampling sources are identified as the UK Urban Pollinators Project (UPP) or additional sampling in the country indicated. Site numbers refer to locations mapped in Figure S1. Habitat categories for farm (F), urban (U) and nature reserve (NR) follow those for the UPP (see Baldock et al. 2015). Full specimen metadata are provided in Table S1.

Site	Source	Site name	Habitat	n	Latitude	Longitude
1	UK (UPP)	Bristol	F	5	51°24′16.27″N	002°41′08.51″W
1	UK (UPP)	Bristol	NR	1	51°26′42.69″N	002°38′56.02″W
2	UK (UPP)	Cardiff	F	1	51°29′53.92″N	003°17′31.78″W
2	UK (UPP)	Cardiff	NR	1	51°32′58.37″N	003°22′22.57″W
3	UK (UPP)	Dundee	F	46	56°22′15.84″N	003°05′39.74″W
3	UK (UPP)	Dundee	NR	11	56°23′14.68″N	002°50′31.82″W
3	UK (UPP)	Dundee	U	8	56°27′42.17″N	002°59′58.59″W
4	UK (UPP)	Edinburgh	F	33	55°49′00.95″N	003°03′57.59″W
4	UK (UPP)	Edinburgh	NR	31	55°49′58.24″N	002°59′23.27″W
5	UK (UPP)	Glasgow	F	12	55°54′06.55″N	003°58′36.64″W
5	UK (UPP)	Glasgow	NR	10	55°57′33.88″N	004°19′56.06″W
6	UK (UPP)	Hull	F	6	53°47′54.93″N	000°35′49.58″W
6	UK (UPP)	Hull	NR	43	53°41′46.21″N	000°27′18.38″W
7	UK (UPP)	Leeds	NR	28	53°37′49.69″N	001°29′47.08″W
8	UK (UPP)	London	F	43	51°40′43.23″N	000°08′34.28″W
8	UK (UPP)	London	U	1	51°29′41.45″N	000°25′26.58″W
9	UK (UPP)	Reading	F	62	51°22′25.60″N	000°55′51.85″W
10	UK (UPP)	Sheffield	F	12	53°29′57.70″N	001°31′35.79″W
11	UK (UPP)	Southampton	F	2	51°01′03.93″N	001°28′00.35″W
12	UK (UPP)	Swindon	NR	7	51°26′03.28″N	001°48′31.05″W
12	UK (UPP)	Swindon	U	2	51°33′30.12″N	001°50′07.84″W
4	UK	Edinburgh	F	5	55°48'52.79''N	3°04'05.81''W
4	UK	Edinburgh	NR	5	55°51'16.00''N	3°13'46.61''W
5	UK	Kildonan, South Uist	F	24	57°13'N	7°24'W
13	UK	Birmingham	U	6	52°27'01.46''N	1°43'51.45''W
1	UK	Bristol	U	9	51°23'12.83''N	2°42'39.05''W
14	UK	Inverness	U	3	57°28'39.62"	4°13'07.63"W
15	Austria	Mariahof	F	26	47°05'N	14°23'E
16	Belgium	Louvain-la-Neuve	U	14	50°39'59.28''N	4°37'22.67''E
17	Bulgaria	Sofia	F	13	42°46'N	23°21'E
18	Croatia	Plitvice	F	10	44°53'N	15°36'E
18	Croatia	Otocac	F	5	44°52'N	15°14'E
19	Estonia	Tartu	F	47	58°21'04.4''N	26°36'83.6''E
20	France	Neuville sur Vanne	F	20	48°15'10"N	3°47'12"E
21	France	Vay	F	15	47°31'00.83"N	1°44'21.78"W
21	France	La Grigonnais	F	5	47°31'05.73"N	1°42'08.15"W
21	France	Carquefou	F	5	47°18'50.54"N	1°30'11.22"W
21	France	Blain	F	5	47°29'26.96"N	1°45'08.28"W
22	Germany	Uslar	F	5	51°39'20''N	9°38'26''E
22	Germany	Göttingen-North	F	5	51°32'46''N	9°55'34''E
22	Germany	Waake	F	5	51°33'24''N	10°3'19''E
22	Germany	Göttingen-South	F	5	51°30'13''N	9°54'55E
22	Germany	Einbeck	F	5	51°49'14''N	9°52'11''E
23	Germany	Puch Fürstenfeldbruck	F	14	48°11'16.63"N	11°12'48.97"E
24	Germany	Pommritz Bautzen	F	12	51°09'29.72"N	14°33'58.58"E
22	Germany	Wesendorf Gifhorn	F	12	53°35'32.14"N	10°32'38.29"E

22	Germany	Sohlingan Uslar	F	10	51°39'58.20"N	9°36'58.20"E
25	Hungary	Bukk Montts	F	7	48°06'30''N	20°49'60''E
26	Hungary	Màtrafüred	F	9	47°49'33''N	19°56'67''E
26	Hungary	Szentkut	F	9	47°59'33''N	19°46'33''E
27	Italy	Biancavilla	U	5	37°40'54.45''N	14°54'13.33''E
28	Poland	Warsaw	F	14	52°9'39.93''N	21°3'15.72''E
29	Romania	Dalga	F	7	44°26'N	27°04'E
30	Spain	A Coruña Arins	F	10	42°51'58.60"N	8°29'55.30"W
31	Sweden	Amalienlund Skane	F	5	56°08'36.37"N	13°04'58.57"E
32	Sweden	Hasslösa gird Vinninga	F	4	58°25'01.46"N	13°09'25.81"E
32	Sweden	Kilagarden Skara	F	5	58°21'00.00"N	13°15'00.00"E
33	Sweden	Rinkabyholm Kalmar	F	3	56°38'57.20"N	16°14'27.02"E
33	Sweden	Vingeslätt Kalmar	F	4	56°47'60.00"N	16°18'00.00"E
34	Sweden	Biovklinge Uppsala	F	5	60°02'09.73"N	17°34'41.18"E
34	Sweden	Solna Uppland	F	4	59°30'14.38"N	16°22'46.38"E
34	Sweden	Fålhagen	F	10	59°51'28.27"N	17°38'59.83"E

Table 2. Food-plants of specimens molecular-identified as *Brassicogethes aeneus*. Only records with confirmed plant identification (n=626) are included. Countries are represented by their three letter ISO codes. The right-hand column indicates food-plants also identified by Williams and Free (1978).

Plant family	Plant species	Country (sample size)	IH Williams and Free 1978
ALLIACEAE	*Allium ursinum*	GBR (2)	
APIACEAE	*Heracleum sphondylium*	GBR (8)	X
ASTERACEAE	*Achillea millefolium*	GBR (9)	X
ASTERACEAE	*Bellis perennis*	GBR (1)	
ASTERACEAE	*Brachyglottis* spp.	GBR (4)	
ASTERACEAE	*Carduus nutans*	GBR (3)	
ASTERACEAE	*Cirsium arvense*	GBR (31)	X
ASTERACEAE	*Cirsium palustre*	GBR (8)	
ASTERACEAE	*Cirsium vulgare*	GBR (2)	X
ASTERACEAE	*Crepis vesicaria*	GBR (2)	
ASTERACEAE	*Helminthotheca echioides*	GBR (10)	
ASTERACEAE	*Hieracium* sp.	AUT (1)	X
ASTERACEAE	*Hypochaeris radicata*	GBR (8)	
ASTERACEAE	*Lapsana communis*	BEL (1), GBR (6)	
ASTERACEAE	*Leontodon saxatilis*	BEL (5)	
ASTERACEAE	*Matricaria chamomilla*	GBR (28)	
ASTERACEAE	*Senecio jacobaea*	GBR (3)	X
ASTERACEAE	*Solidago gigantea*	SWE (1)	
ASTERACEAE	*Sonchus arvensis*	GBR (10)	
ASTERACEAE	*Sonchus asper*	GBR (3)	X
ASTERACEAE	*Sonchus palustris*	BEL (4)	
ASTERACEAE	*Tanacetum parthenium*	GBR (12)	
ASTERACEAE	*Taraxacum* agg.	AUT (1), HUN (3), GBR (26)	X
ASTERACEAE	*Tripolium pannonicum*	SWE (1)	
BRASSICACEAE	*Aubrieta* sp.	GBR (1)	
BRASSICACEAE	*Brassica napus* sbsp. *oleifera*	BGR (12), EST (38), FRA (40), GER (67), POL (5), ROM (7), SWE (29), GBR (76)	
BRASSICACEAE	*Sinapis alba*	FRA (5), POL (7)	
BRASSICACEAE	*Sinapis arvensis*	GBR (1)	X
FABACEAE	*Genista tinctoria*	ITA (1)	
FABACEAE	*Lupinus luteus*	GER (6)	
LILIACEAE	*Gagea lutea*	HUN (1)	
RANUNCULACEAE	*Ficaria verna*	HUN (13)	
RANUNCULACEAE	*Ranunculus acris*	BEL (4), GBR (18)	
RANUNCULACEAE	*Ranunculus arvensis*	AUT (1)	
RANUNCULACEAE	*Ranunculus flammula*	GBR (1)	
RANUNCULACEAE	*Ranunculus repens*	GBR (52)	X
ROSACEAE	*Filipendula ulmaria*	GBR (3)	
ROSACEAE	*Rosa* sp.	GBR (2)	X
ROSACEAE	*Rubus fruticosus* agg.	GBR (42)	X
RUBIACEAE	*Galium uliginosum*	GBR (1)	

Supplementary Material

Supplement 1

Table S1. Full metadata and accession numbers for all specimens used in analyses, including previously published sequences.

Supplement 2

Table S2. Levels and sample sizes for the hierarchical AMOVA analyses of samples identified as *Brassicogethes aeneus*.

Supplement 3

File S1. Keyhole Markup Language (.kmz) format file of sampling locations and associated metadata suitable for viewing in Google Earth. Map data ©2016 GeoBasis-DE/BKG (©2009), Google Imagery ©2016 NASA, TerraMetrics.

Supplement 4

Figure S1. Sampling locations for pollen beetles in this study. Sites 1-34 refer to site names and metadata in Table 1, while sites 35-40 identify site locations for published sequences. The colour for each location symbol identifies the European regional grouping used in AMOVA analysis of

Brassicogethes aeneus (red=Scotland, green=England and Wales, yellow=France/Belgium/Germany, purple=Scandinavia and the Baltic, pink= Southern Europe).

Supplement 5

Figure S2. Food-plant associations of sampled beetles. Full metadata for each specimen are provided in Table S1. (a) Numbers of species in each plant family from which adult beetles were collected in this study, and (b) the numbers of adult beetles collected from each plant family across the whole study. In (a) and (b), coloured bars for each plant family show (from left) sampling for all beetles field-identified as pollen beetles, beetles BLAST-identified as Meligethinae, and beetles sequence-matched with *Brassicogethes aeneus*. (c) Numbers of adult beetle (field-identified as Meligethinae) collected from each plant family in farmland and nature reserve habitats in the UK Urban Pollinators Project.

Supplement 6

Figure S3. Phylogenetic relationships between the full set of 241 unique CO1 haplotype sequences for published Meligethinae and newly sampled specimens identified by BLAST search as ≥98% similar to Meligethinae. The tree shown is a Bayesian majority rule consensus tree inferred using MrBayes (Ronquist and Huelsenbeck 2003) using a GTR+I+G model of sequence evolution selected using MrModeltest (Nylander 2004), and rooted with a published sequence for *Kateretes rufilabris* (Genbank accession DQ221966). Numbers at nodes indicate posterior probability support. To simplify presentation, where multiple copies of a haplotype were sampled we illustrate only one per habitat type (farm, urban or nature reserve) for UK Urban Pollinators Program sites (taxon

names all in red), and one copy per country for sites outside the UK. Coloured circles by taxon names show the European regions used in AMOVA analyses for *Brassicogethes aeneus* and *B. viridescens*.

Supplement 7

Figure S4. Variation in the number of Meligethinae MOTUs (Molecular Operational Taxonomic Units) resolved in our dataset as a function of percentage sequence divergence, analysed using either jMOTU (panel a) or ABGD (panel b).