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Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae)

Michael Kuhlmann[a,*], George R. Else[b], Anna Dawson[c], Donald L.J. Quicke[b,c]

[a]*Institute of Landscape Ecology, University of Münster, Robert-Koch-Str. 26, 48149 Münster, Germany*
[b]*Department of Entomology, Natural History Museum, London SW7 5BD, UK*
[c]*Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK*

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Abstract

This paper examines a cryptic species complex comprising three nominal bee species: *Colletes halophilus*, *Colletes hederae*, and *Colletes succinctus*. Multiple individuals of each were sequenced for four gene fragments: mitochondrial cytochrome oxidase 1 (CO-1), internal transcribed spacer 2 (ITS-2) and flanking regions, elongation factor 1 alpha (EF-1α), and 28S. In addition, the distribution patterns and phenology of all species were examined. Fixed substitutions distinguishing the three species are present in EF-1α and ITS-2. Their distribution patterns differ clearly: *Colletes halophilus* is endemic to coastal habitats of the southern North Sea and the English Channel; *Colletes hederae* occurs from Slovenia in the southeast to southern England in the northwest; the range of *Colletes succinctus* covers most of Europe and reaches western Kazakhstan. Where the three species occur jointly in western Europe, their flight activities differ significantly. The genetic distinctions and the differences in distribution, phenology and flower specialisation clearly support their status as distinct species.

Keywords: *Colletes*; Sibling species; Genetic differences; Biogeography; Phenology

Introduction

The *Colletes succinctus* group of mining bees is characterised by the synapomorphy of a pair of prominent, deep lateral pits on sternite 6 of the male. It comprises at least 12 species in two subgroups, of which three species occur in western Europe (Kuhlmann 2000, 2003; Dubitzky and Kuhlmann 2004). Females in the group can be recognised by the translucent reddish-orange colour of the posterior margin (marginal area) of the basal tergite. The species occurring in western Europe are *Colletes halophilus* Verhoeff 1944, *Colletes hederae* Schmidt and Westrich 1993, and *Colletes succinctus* (Linnaeus, 1758). In addition, *Colletes collaris* Dours, 1872 and *Colletes brevigena* Noskiewicz, 1936 are found sporadically in southern regions. Especially the former three are extremely closely related and are very difficult to distinguish on morphological characters, the differences being very subtle. However, each of these species has specific habitat requirements and floral preferences which in most cases allow accurate identification. Nevertheless, the taxonomic status of the three species has been unclear.

*Corresponding author.
E-mail address: kuhlmmi@uni-muenster.de (M. Kuhlmann).

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The identification of cryptic (= sibling) species can sometimes be aided by differences in geographical range, phenology, ontogeny or host use, or by proof of reproductive isolation under laboratory conditions (Pinto et al. 1991; Page and Holmes 1998). For example, studies of life history events have proven useful in distinguishing between two 'strains' of *Bracon hebetor*, but there is little doubt that these are genetically isolated and ought to be regarded as separate species. Differences in percent of pre-oviposition paralysis, number of eggs laid per day, and egg-to-adult survival have led to positive differentiation of the two strains (Heimpel et al. 1997). However, it also happens that organisms are classified as different species based upon very small differences in behaviour, morphology or plant choice, for example, when they are simply expressing different environmental effects or genetic polymorphisms. Molecular analysis can be employed to help solve such problems. Analysis of DNA sequences offers potentially huge data sets and can discern relatively accurately the true identity of an organism and whether it is distinctly a species in its own right.

Whether any gene fragments can be used reliably and economically to distinguish closely related species is central to the currently popular idea of DNA barcoding (see Hebert et al. 2003a, b), and genes are becoming ever increasingly used to aid in the diagnosis of new species where morphological characters are either unreliable or hard to evaluate (Quicke 2003; Gullan et al. 2003). The nuclear ribosomal internal transcribed spacer (ITS-2) has proven phylogenetically informative in separating two cryptic species, and sometimes populations, in several parasitoid hymenopterans, e.g. of the encyrtid *Ageniaspis* (Alvarez and Hoy 2002), the braconid *Monilobracon* (Quicke et al., unpubl. data) and the cynipid *Alloxysta* (van Veen et al. 2004).

In the present paper we examine four gene fragments of CO-1, ITS-2 and flanking regions, EF-1α, and 28S, and analyse distribution patterns and flight activity of all three species.

Taxonomic situation and bionomics of species of the *Colletes succinctus* group

Colletes halophilus

The name was originally established for a subspecies of *Colletes succinctus* (Verhoeff 1944), but later authors (e.g. Yarrow 1954; Guichard 1974; Schwarz et al. 1996) have used it at species level. According to some workers there are very slight differences in the punctation of the basal gastral tergites between *halophilus* and *succinctus*, but these are difficult to appreciate if only one specimen is available for study. The most reliable character, however, is the form of the outer, more heavily sclerotised portion of the galea. This is highly polished (shining), without microsculpture of any kind, in both sexes of *Colletes succinctus*. In *Colletes halophilus* this part of the galea is dull owing to extensive microreticulation. The species is very difficult to distinguish from *Colletes hederae*.

Both sexes mainly visit the flowers of *Aster tripolium*, though other, unrelated, flowers are also visited. *Aster tripolium* is the main pollen source for this bee in Britain (C. O'Toole, pers. comm. 2003) but it is likely to be oligolectic on other Asteraceae (Westrich 1989). In both Britain and mainland Europe, *Colletes halophilus* is associated with the margins of salt marshes and, occasionally, other coastal habitats. Its distribution is restricted to the southern North Sea and the English Channel. Generally, the species nests in dense aggregations (Guichard 1974; authors' pers. obs.) in both level and sloping soil, especially that with a high sand content. The nest architecture has been illustrated by O'Toole and Raw (1991). Nests are attacked by the bee cleptoparasites *Epeolus variegatus* (Linnaeus, 1758) and, only in the south-west Netherlands, also by *Epeolus tarsalis* Morawitz, 1874 (Peeters et al. 1999). *Colletes halophilus* flies as a single brood from mid August to mid October, rarely early November.

Colletes hederae

Formerly misidentified as *Colletes halophilus*, the species was described only recently (Schmidt and Westrich 1993). Both sexes are extremely similar to those of *Colletes halophilus*. There are slight differences in the punctation on gastral tergites 1 and 2 and, in the females, there are also differences in the form of the longitudinal striae on the clypeus: in *Colletes hederae* these turn inwards apically towards the midline, whereas in *Colletes halophilus* they do not. The apical hair bands on gastral tergites 1–5 of the female and 1–6 of the male are distinctly yellowish in freshly emerged *Colletes hederae* but white in *Colletes halophilus*. The average body length for both sexes of *Colletes hederae* is greater than that in its sibling species, though there is an overlap.

Colletes hederae is sporadically distributed in Europe, being known from Croatia in the southeast to southern England in the northwest (Bischoff et al. 2005). In most English and Channel Island sites where it occurs the populations are very large, some in the order of tens of thousands of individuals (e.g. between Worth Matravers and Winspit, Dorset, England). It is usual to see numerous specimens flying about the pollen and nectar source. As its specific name implies, this bee restricts its pollen visits entirely to the flowers of *Hedera helix* (Araliaceae) (Schmidt and Westrich 1993; Bischoff et al. 2005). Both sexes also fly to the same flowers for nectar, though in Jersey, Channel Islands, some males have

been observed to visit *Calluna vulgaris* for the same purpose (B. Bolton, pers. comm. 2003). The species nests in large aggregations, including the margins of footpaths, vertical cliff surfaces and roadside banks, and the sloping lawns of some private gardens. In southern Europe nests are attacked by the bee cleptoparasite *Epeolus cruciger* (Panzer, 1799) (A. Müller, A. Gogala, pers. comm. 2004). The flight period extends from early September to November.

Colletes succinctus

Both sexes of this species are readily distinguished from those of its two close relatives by the polished surface (devoid of microsculpture) of the outer, thickened portion of the galea.

This species is the most widespread member of its species group in western Europe. This bee is mostly found on lowland heaths, and partly on moorland. Some populations occur on maritime dunes and beaches. Males are often encountered in large numbers, skimming low over flowering *Erica* species and *Calluna vulgaris* (Ericaceae), these being the principal pollen sources of the females, though they utilise the same plants for nectar (Westrich 1989). However, some populations seem to confine visits to yellow Asteraceae flowers (especially *Senecio jacobaea*) (Perkins 1945; Edwards and Telfer 2001), and these may represent a different ecological race, though morphological characters are apparently identical to those bees which exploit only Ericaceae flowers for pollen and nectar. Several females were found visiting *Hedera helix* flowers on the Isle of Man in 2002 (S. Crellin, pers. comm. 2003) and more recently on the Isles of Scilly (I. Beavis, pers. comm. 2004). The females nest in large, dense aggregations in northern Britain but usually in very small ones (often difficult to locate) in southern England and other parts of western Europe. A nest of this species has been illustrated by O'Toole (1986). As in all *Colletes* species, the cell walls are thin and transparent, resembling cellophane in appearance (they are derived from a secretion of the abdominal Dufour's gland) (Bergström and Tengö 1974, 1978). The flight period begins in mid July and ceases in late September or October. Nests are subject to attack by the cleptoparasitic bee *Epeolus cruciger* (Westrich 1989).

Material and methods

Molecular data

Bees for this study were collected by G.E. or generously provided by colleagues (see Acknowledgements). Specimens used for sequencing were primarily preserved in 95% ethanol, but recently collected pinned specimens (< 5 years old) were also used. The ingroup is composed of *Colletes halophilus*, *Colletes hederae* and *Colletes succinctus*. The outgroup includes other species of the *Colletes succinctus* group (*Colletes brevigena*, *Colletes collaris* and *Colletes intricans* Spinola, 1838) and the unrelated *Colletes acutus* Pérez, 1903 and *Colletes wolfi* Kuhlmann, 1999. The taxa included in this study, collecting data, and GenBank accession numbers are listed in Tables 1 and 2. Voucher specimens of all individuals used for DNA sequencing are deposited in the Natural History Museum, London.

DNA extractions were carried out by placing the dried samples in 50 µl of 5% (w/v) Chelex 8 (Bio-Rad, Inc.) containing 12 µg/ml proteinase K, followed by digestion for approximately 2 h at 55–60 °C. Proteinase K was then heat-inactivated at 96 °C for 15 min. Samples were vortexed for approximately 10 s and the Chelex pelleted by centrifugation at 13,000*G* for approximately 30 s prior to removal of 2 µl of supernatant as template for PCR reactions. CO-1 primers were designed by Folmer et al. (1994) (LCO 5′GGT CAA CAA ATC ATA AAG ATA TTG G3′; HCO 5′TAA ACT TCA GGG TGA CCA AAA AAT CA3′). The primers used for EF-1α were from Sanchis et al. (2001) (5′GATGGCACGGAGACAAC-ATG 3′; 5′ACACCAGTTTCAACACGACC 3′), and gave a fragment of c. 400 bp. Another reverse primer (5′CCATTGCTGATTTGTCCAGGGTGG 3′) was also used with the same forward primer to obtain some longer sequences of c. 800 bp, but with less success. The ITS-2 primers used were based on Zhu and Greenstone's (1999) 58S forward primer but shortened (5′ TGT GAA CTG CAG GAC ACA TG 3′), and reverse shortened from that of Onyabe and Conn (1999) (5′ATG CTT AAA TTT AGG GGG T 3′). The 28S primers were designed by Belshaw and Quicke (1997) (fwd: 5′GCG AAC AAG TAC CGT GAG GG3′) and Mardulyn and Whitfield (1999) (rev: 5′TAG TTC ACC ATC TTT CGG GTC CC'3). PCR reactions were carried out in a 25 µl final volume using pure *Taq* ready-to-go PCR beads (Amersham Biosciences). The PCR programme, used for all amplifications, had an initial 4 min denaturation at 80 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 90 s. A 10 min extension period following the final cycle was added in all cases. PCR products were purified using the wizard SV gel and PCR clean-up system (Promega, Inc.), and then sequenced in both directions using dideoxy terminator cycle sequencing (Applied Biosystems, Inc.) with an ABI 3700 automated DNA sequencer.

Biogeography and phenology

Information on the distribution and phenology of *Colletes halophilus*, *Colletes hederae* and *Colletes succinctus* was extracted from the *Colletes* data bank of

Table 1. Specimens studied, collecting localities, dates, and GenBank accession numbers for CO-1, ITS-2, and EF-1α

No.	Provenance	Coll. date	GenBank accession numbers		
			CO-1	ITS-2	EF-1α
Colletes halophilus					
6	GB, Fingringhoe Wick, Nat. Reserve	ix.2004	DQ085526	DQ085588	—
7	GB, Norfolk, Holmes Dunes Nat. Reserve	7.ix.2002	DQ085527	DQ085589	DQ085555
8	GB, Essex, East Tilbury silt lagoons	15.ix.2002	DQ085528	DQ085590	DQ085556
9	GB, Kent, V616, Cliffe marshes	16.ix.2002	DQ085529	DQ085591	—
B1	F, Baie l′Authie	3.ix.2004	DQ085530	DQ085592	—
B2	F, La Slack	3.ix.2004	DQ085531	DQ085593	—
B3	F, Le Crotoy	3.ix.2004	DQ085532	DQ085594	—
B5	F, Baie de Somme	3.ix.2004	DQ085533	DQ085596	—
B8	F, Baie de Somme	3.ix.2004	—	DQ085598	DQ085559
B6	F, La Slack	3.ix.2004	DQ085534	DQ085595	DQ085557
B7	NL, Voorne	26.viii.2004	DQ085535	DQ085597	DQ085558
B10	NL, Ellewoutsdijk	9.ix.2004	DQ085536	DQ085599	DQ085560
B11	NL, Goedereede, Kwade hoek	17.ix.2004	DQ085537	DQ085600	DQ085561
B13	NL, Krabbendijke, Rattenkaai	16.ix.2004	DQ085539	DQ085602	DQ085563
B14	NL, Terneuzen, Paulinaschorren	16.ix.2004	—	DQ085603	DQ085564
B15	NL, Vlissingen, Hooge Plaaten	16.ix.2004	—	—	DQ085565
B18	NL, Nieuwevliet, Verdronken Zwarte Polder	18.ix.2004	DQ085541	DQ085606	DQ085568
B19	NL, Emmadorp, Verdr. Land van Saeftinghe	ix.2004	DQ085542	DQ085607	—
B23	NL, Terneuzen	9.ix.2004	DQ085543	DQ085610	DQ085570
B12	B, Knokke Heist, het Zwin	16.ix.2004	DQ085538	DQ085601	DQ085562
B16	B, IJzermonding	18.ix.2004	DQ085540	DQ085604	DQ085566
B17	B, Baai van Heist	18.ix.2004	—	DQ085605	DQ085567
B20	D, 5 km S Emden	17.ix.2004	—	DQ085608	DQ085569
Colletes hederae					
1	GB, Dorset, Worth Matravers	ix..2002	DQ085513	DQ085578	—
2	GB, Dorset, West Lulworth	28.ix.2002	DQ085514	DQ085579	—
3	GB, Channel Islands, Jersey, St. Aubain	ix.2002	DQ085515	DQ085580	—
B21	GB	ix.2004	—	DQ085609	DQ085552
B24	GB, Dorset, Swanage Station	16.x.2004	DQ085518	DQ085582	DQ085554
B22	NL, Terneuzen	1.x.2004	DQ085517	DQ085581	DQ085553
B4	F, Le Crotoy	3.ix.2004	DQ085516	—	DQ085551
Colletes succinctus					
4	GB, Surrey, Pirbright Ranges	22.viii.2002	DQ085519	DQ085583	DQ085571
10	GB, Cumbria, Sunbiggin Tarn	1.ix.2002	DQ085520	DQ085584	DQ085572
11	GB, Dorset, Canford Heath	21.viii.2002	DQ085521	DQ085585	DQ085573
12	GB, Dorset, Canford Heath	21.viii.2002	DQ085522	DQ085586	DQ085575
13	GB, Dorset, Broadstone garden	17.viii.2002	DQ085523	—	DQ085574
38	N, Vay Sogne, Hellevika	25.viii.2000	DQ085525	DQ085587	—
37	D, Dorsten	4.ix.1999	DQ085524	—	—
Colletes brevigena					
	GR, Crete, Spili/Gerakari	1.vi.2002	DQ085546	—	—
Colletes intricans					
	TN, South of M′Saken	21.v.1999	DQ085545	—	—
Colletes collaris					
	RUS, near Kysyl	16.viii.1998	DQ085544	—	—
Colletes acutus					
	I, Sardegna, 20 km SSE Siniscola	25.iv.2000	DQ085548	DQ085611	DQ085576
	E, Coto Doñana National Park	15.iv.2003	DQ085549	—	—
Colletes wolfi					
	I, Piemonte, Roccaverano	vi.2004	DQ085550	—	DQ085577

Table 2. Specimens studied, collecting localities, dates, and GenBank accession numbers for 28S

Species	Provenance	Coll. date	Acc. no.
Colletes halophilus	GB, Essex, East Tilbury silt lagoons	15.ix.2002	DQ085508
Colletes hederae	GB, Dorset, Worth Matravers	ix..2002	DQ085511
Colletes succinctus	N, Vay Sogne, Hellevika	25.viii.2000	DQ085507
	D, Dorsten	4.ix.1999	DQ085506
Colletes brevigena	GR, Crete, Spili/Gerakari	1.vi.2002	DQ085509
Colletes intricans	TN, South of M′Saken	21.v.1999	DQ085510
Colletes wolfi	I, Piemonte, Roccaverano	vi.2004	DQ085512

M. Kuhlmann (unpublished), which is based on published information and predominantly examination of numerous museum and private collections. For the distribution of *Hedera helix* (Araliaceae), *Calluna vulgaris* and *Erica* species (Ericaceae), the respective pollen sources of *Colletes hederae* and *Colletes succinctus*, Meusel (1978) was consulted.

Data about the flight periods is based solely on records from the UK and The Netherlands, the latter a region where all three species occur together. Records of males and females were summarised in 10-day periods (decades), and their phenological pattern statistically tested for differences using the *G*-test.

Results and discussion

Molecular data

Numerous substitutions and indels show the three *Colletes succinctus* group species to form a closely related group separate from other *Colletes*. From the four investigated gene fragments, four fixed, single base pair differences were found separating one or other of the three *succinctus* group species, and two other positions showed marked variation in allele frequency. The differences observed are summarised in Table 3 and Fig. 1.

The ITS-2 sequences were identical for all individuals of the *succinctus* group, except for a single position near the middle of the sequenced fragment. At this position, six out of six *Colletes hederae* and 22 out of 22 *Colletes halophilus* have an A, whereas five out of five *Colletes succinctus* have a G.

Three positions in the EF-1α fragment showed fixed differences between one of the three species and the remaining two. At the first position towards the 5′ end, four out of four sequenced *Colletes hederae* have a T, whereas four out of four *Colletes succinctus* and 12 out of 12 *Colletes halophilus* have a G. The second fixed difference in EF-1α was located near the middle of the sequenced fragment: here, four out of four *Colletes hederae* and 12 out of 12 *Colletes halophilus* have a C, whereas three out of three *Colletes succinctus* have a T. Towards the 3′ end of the EF-1α a third fixed difference was identified, but the numbers of individuals sequenced were rather small: *Colletes hederae* has a G ($n = 3$ with one individual each from England, The Netherlands and France), whereas *Colletes succinctus* ($n = 2$) and *Colletes halophilus* ($n = 10$, including individuals from the UK, Germany, Belgium and The Netherlands) have a T.

No fixed differences were found in the CO-1 gene fragment, but at position 341 (corresponding to nucleotide 346 in the NCBI *Apis mellifera* reference sequence [NC_001566]), six out of six *Colletes hederae* (from UK, The Netherlands and France), seven out of seven *Colletes succinctus*, and one individual out of 18 *Colletes halophilus* have a G, whereas the remaining 17 *Colletes halophilus* have an A. Also, at position 119 (corresponding to nucleotide 124 in the NCBI *Apis mellifera* reference sequence [NC_001566]) of the CO-1 fragment, *Colletes hederae* has a fixed G ($n = 4$), and *Colletes succinctus* an A ($n = 6$), but this position is polymorphic for A and G in *Colletes halophilus*. No differences were found in the 28S gene fragment.

Greater intraspecific variation was observed among the sequenced *Colletes halophilus* individuals, but we had far more representatives of this species in our data set. Of note is a polymorphism for an indel (and an associated substitution) in the EF-1α gene fragment, with two bees (B6 from France and B12 from Belgium) differing from all the others. A four base pair deletion in the EF-1α fragment was also present in one of the five sequenced *Colletes succinctus*.

Biogeography

The distribution areas of all three species are of very different extension and position, with those of *Colletes halophilus* and *Colletes hederae* lying entirely within that of *Colletes succinctus*. All species occur jointly in the coastal areas along the English Channel (SW England, SW Netherlands, Belgium, NW France).

Colletes halophilus has the smallest distribution. It is an endemic of coastal areas of the southern North Sea and along the English Channel (Fig. 2), where it is largely restricted to salt marshes. This species is a

Table 3. Sequence fragments showing distinguishing substitutions for species of the western European *Colletes succinctus* species complex and outgroup taxa

Gene	Species	Sequence						
CO-1 region 1			•			•	•	
	hederae	TTATA	TTTTA	TTTTT	GCTAT	**G**TGAA	CTGG**A**	A
	succinctus	TTATA	**C**TTTA	TTTTT	GCTAT	**G**TGAA	CTGG**A**	A
	halophilus	TTATA	TTTTA	TTTTT	GCTAT	**G**TGAA	CTGG**A**	A
	acutus	TTATA	TTTTA	TTTTT	GCAAT	ATGAA	CTGGT	A
CO-1 region 2		•	• •	•	•	••	•	•	•
	hederae	TAGAG	GAACT	GGATG	G**A**CT**A**	T**T**TAT	CCTCC	**T**
	succinctus	TAGAG	GAACT	GGATG	G**A**CT**A**	T**T**TAT	CCTCC	**T**
	brevigena	TAGAG	GAACT	GGATG	G**A**CT**A**	T**T**TAT	CCTCC	**T**
	intricans	TAGAG	GAACT	GGATG	**R**ACT**A**	T**T**TAT	CCTCC	**T**
	halophilus	TAGAG	GAACT	GGATG	AACT**A**	T**T**TAT	CCTCC	**T**
	collaris	**A**AGAG	G**T**ACT	GGATG	AAC**A**G	T**T**TAT	CC**A**CC	**T**
	acutus	TAGAG	GAAC**A**	GG**T**TG	AACTG	T**T**TAT	CCTCC	**T**
	wolfi	TAGAG	GAACT	GGATG	AACTG	TATAT	CCTCC	A
EF-1α intron, region 1				•				
	hederae	AACGT	AAAGA	**T**AATA	AAGCG	GAAGG	AAAAT	G
	succinctus	AACGT	AAAGA	GAATA	AAGCG	GAAGG	AAAAT	G
	halophilus	AACGT	AAAGA	GAATA	AAGCG	GAAGG	AAAAT	G
	brevigena	AACGT	AAAGA	GAATA	AAGCG	GAAGG	AAAAT	G
EF-1α intron, region 2					•			
	hederae	GTCTG	TTGAA	ATGCA	CCACG	AAGCA	TTGCA	A
	succinctus	GTCTG	TTGAA	ATGCA	**T**CACG	AAGCA	TTGCA	A
	halophilus	GTCTG	TTGAA	ATGCA	CCACG	AAGCA	TTGCA	A
EF-1α intron, region 3				•		•		
	hederae	CAAAT	TCTGG	GCAAA	TT**G**AA	CTATA	GATCA	T
	succinctus	CAAAT	TCTG**A**	GCAAA	TTTAA	CTATA	GATCA	T
	halophilus	CAAAT	TCTGG	GCAAA	TTTAA	CTATA	GATCA	T
ITS-2					•	••		
	hederae	TTTGA	TGGCA	ATAAA	**AT**AAC	CACCG	TGGGG	C
	succinctus	TTTGA	TGGCA	AT**G**AA	**AT**AAC	CACCG	TGGGG	C
	halophilus	TTTGA	TGGCA	ATAAA	**AT**AAC	CACCG	TGGGG	C
	brevigena	TTTGA	TGGCA	ATAAA	−−AAC	CACCG	TGGGG	C

specialised visitor of Asteraceae with a strong preference for *Aster tripolium*. As Asteraceae are ubiquitous they are not represented in Fig. 2. In Britain, *Colletes halophilus* is confined to south-east England, from Humberside south to Kent and west to east Dorset. A British distribution map has been provided by Edwards (1997). The distribution on mainland Europe ranges from the coasts of north-west France (southernmost record from Le Crotoy), Belgium, The Netherlands (Manning 1955) to north-west Germany with the easternmost record from the east Frisian island of Mellum.

There are no recognisable reasons for the very restricted distribution of *Colletes halophilus* and the fact that it is missing in large suitable areas in the neighbourhood of inhabited habitats (e.g. the salt marshes south and east of Mellum (V. Haeseler, in litt. 2004) and west of Hengistbury Head near Bournemouth). This is without parallel among western European bees and can only be compared with the similar but more eastward extended distribution pattern of the nominate form of the bumble bee *Bombus c. cullumanus* (Kirby, 1802). This very rare and nearly extinct species is largely restricted to coastal areas and is recorded from south-east England, The Netherlands, Germany, Denmark and southern Sweden (Løken 1973). In *Colletes halophilus* this pattern can be intepreted in two ways: it might be the result of recent speciation and perhaps an unfinished dispersal process or it could be a relict of postglacial immigration and following extinction in its glacial refuge. The latter would be similar to the north-west European coastal populations of *Colletes impunctatus* Nylander, 1852 and *Colletes floralis* Eversmann, 1852. Both species normally only occur in alpine

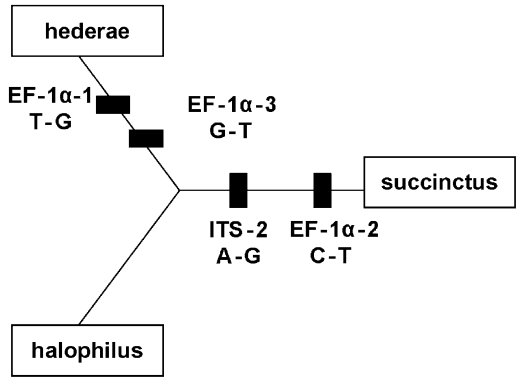

Fig. 1. Fixed genetic differences and relationships of *Colletes succinctus*, *Colletes hederae* and *Colletes halophilus*.

Fig. 2. Distribution of *Colletes halophilus*.

environments or in the boreal climate zone but seem to form relict populations in the similar type of open dynamic habitat of the coastal environment. It is interesting that one of the cuckoo bees of *Colletes halophilus*, *Epeolus tarsalis*, is restricted to the delta area of the SW Netherlands (Peeters et al. 1999; C. Jacobusse, pers. comm. 2005). This cuckoo bee is originally a parasite of the closely related *Colletes collaris* (Pittioni 1945) and like the latter has a transpalaearctic distribution (M. Schwarz, in litt. 2004). *Epeolus tarsalis* seems to be not very well adapted to its host *Colletes halophilus*: despite very large nesting aggregations it is extremely rare and is only known from four sites (C. Jacobusse, pers comm. 2005). This may support the hypothesis of a recent origin of *Colletes halophilus* and an insufficiently adapted cuckoo bee that was not able to follow its host after range expansion.

The distribution of *Colletes hederae* (Fig. 3) was first described in Bischoff et al. (2005). The range extends from central Italy, Sardinia and north-east Spain in the south to south-west Germany, Netherlands and southern parts of Great Britain in the north. The east-west extension is from Slovenia to the English Channel. It has long been known from all the larger Channel Islands (Guernsey, Herm, Jersey, and Sark), where it was originally misidentified as *Colletes succinctus* (Richards 1979). A distribution map of this species on the Channel Islands has been presented by Edwards and Telfer (2001). *Colletes hederae* seems to have colonised southern England very recently, having been first found there in Dorset in 2001 (Cross 2002). It is currently known from Dorset (Mudeford to Weymouth and Portland), Hampshire (Barton on Sea east to Hayling Island and north to Martin Down), East Sussex (Hastings), Somerset (Bruton), Wiltshire (Cranborne chase), and east Devon (Beer to Branscombe, East Prawle). A few sites in Dorset are some distance inland (Corfe Castle village, Wareham, Wool, Milborne St. Andrews). According to Lattin (1967), *Colletes hederae* represents an adriato-mediterranean distribution type. All known localities of this species fall within the distribution of *Hedera helix*, but its range is much smaller than that of its host plant (Fig. 2). *Hedera helix* occurs from North Africa in the south to southern Scandinavia in the north, and from the Canaries and the United Kingdom in the west eastward to the coast of the Black Sea (Meusel 1978).

The distribution pattern of *Colletes hederae* can be interpreted as the result of the postglacial recolonisation of parts of Europe from an Italian refuge (Lattin 1967; Hewitt 1999). The bee's restricted distribution compared with that of its host plant may indicate a relatively recent origin of *Colletes hederae* and an unfinished dispersal process, as evidenced by the recent colonisation of southern England. This hypothesis is supported by the fact that *Colletes hederae* is only known to be attacked by the cuckoo bee *Epeolus cruciger* south of the Alps (A. Müller, A. Gogala, pers. comm. 2004). It is likely that *Epeolus cruciger* living on *Colletes hederae* is a specific 'host race' of this widely distributed species in Europe that has evolved in the glacial refuge and was not able to follow its host during postglacial range expansion.

Colletes succinctus is widely distributed in Europe (Fig. 4). It has been recorded from Portugal and Ireland to western Kazakhstan. The latitudinal distribution is obviously limited by climatic factors. In the north (Scandinavia) *Colletes succinctus* rarely exceeds 61°N, in the south it is sporadically found in the northern part of the Mediterranean Basin, but lacking in North Africa where it is replaced by the close relative *Colletes intricans* (Kuhlmann 2003). Within these limits the distribution of *Colletes succinctus* coincides with those of its host plants *Calluna vulgaris* and *Erica* species (Fig. 4). Records of *Colletes succinctus* apparently outside the distribution range of *Calluna vulgaris* in

Fig. 3. Distribution of *Colletes hederae* (solid circles) and its host plant, *Hedera helix* (shaded area).

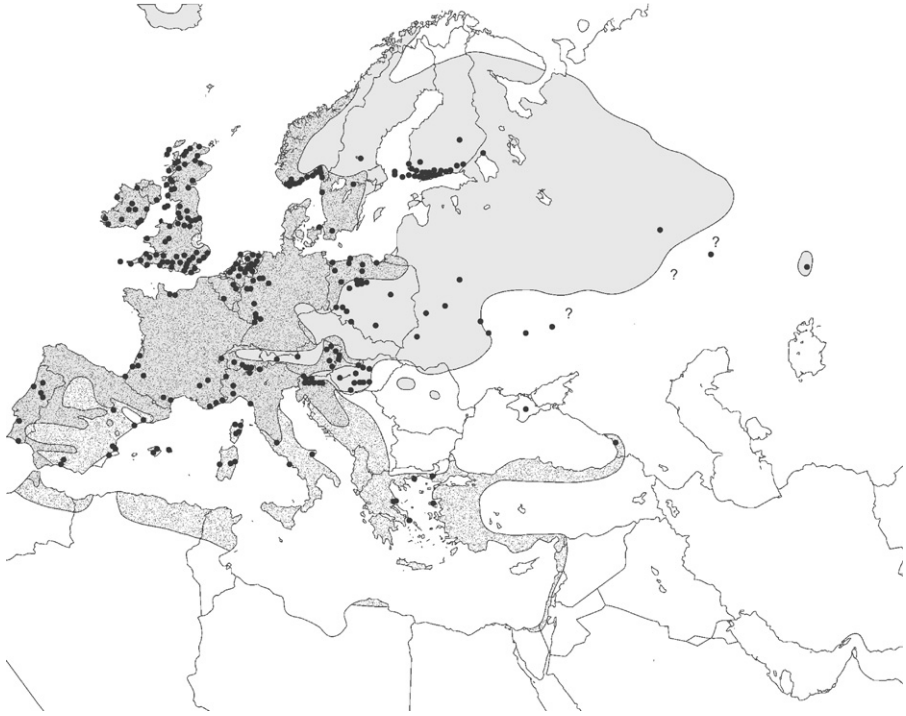

Fig. 4. Distribution of *Colletes succinctus* (solid circles) and its host plants, *Calluna vulgaris* (shaded area; ? = uncertain or isolated occurrence) and *Erica* spp. (dotted area).

the northern Ukraine are caused by insufficient data from this area and scattered, isolated populations of heath not representable in the map. The single, isolated record from the Crimean Peninsula (Osychnyuk 1970) is far outside the known distribution of the host plants and is probably a misidentification. As with the host plants, the distribution of *Colletes succinctus* and its cuckoo bee *Epeolus cruciger* is largely congruent (M. Schwarz, in litt. 2004).

The congruence of distribution patterns between *Colletes succinctus* and its cuckoo bee and host plants forms a sharp contrast to the situation in *Colletes*

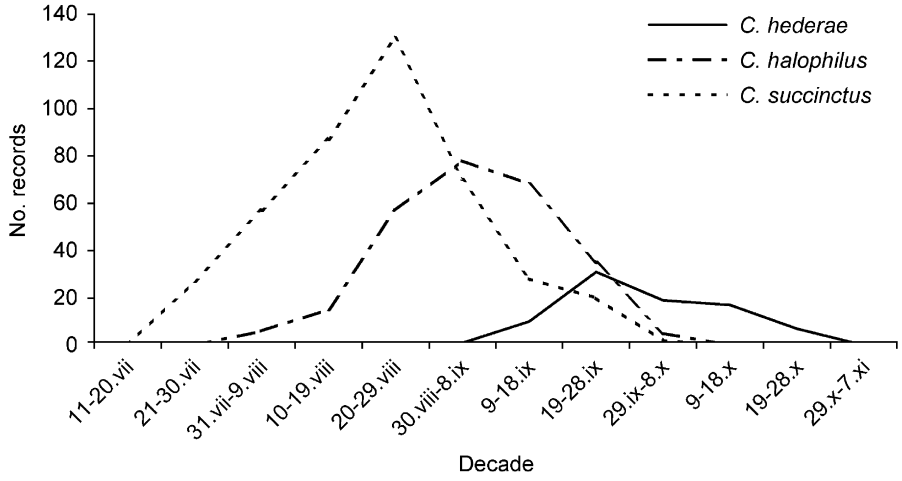

Fig. 5. Phenology of *Colletes succinctus*, *Colletes hederae* and *Colletes halophilus* based on records from The Netherlands and Britain.

halophilus and *Colletes hederae*. It can be interpreted as a comparatively older system in which the species have reached a higher degree of adaptation.

Phenology

The flight activity of the three species shows a characteristic pattern (Fig. 5). The earliest-flying bee is *Colletes succinctus*, which can be found from early July to the beginning of October, with a maximum at the end of August ($n = 421$). The activity of *Colletes halophilus* starts about 10 days later and as in the former species ends at the beginning of October, with the maximum in the beginning of September ($n = 264$). The difference in number of records per decade is highly significant between both species ($G = 157.418$, FG = 7, $p < 0.001$). *Colletes hederae* is one of the latest active bee species in western Europe. Its flight activity starts in the beginning of September, about a month later than *Colletes halophilus*, and can last until the beginning of November. The maximum number of records was made at the end of September ($n = 89$). The flight activities of *Colletes halophilus* and *Colletes hederae* differ significantly ($G = 89.153$, FG = 8, $p < 0.001$). In Terneuzen (south-west Netherlands), where *Colletes halophilus* and *Colletes hederae* occur in close proximity, direct observations confirmed that the former species is active about 3 weeks earlier than the latter (L. Calle, pers. comm. 2005). Most female bees appear to mate only once and are only receptive during a short period around emergence time (Roubik 1989). This is also true for *Colletes* (Bergström and Tengö 1978; authors' pers. obs.). Thus, due to their differences in phenology and habitat requirements, all three species are largely separated temporally as well as spatially, and thereby reproductively isolated. Males of each of the three sibling species have hardly any chance to meet and copulate with receptive females of the other species. Moreover, first experiments in Terneuzen showed that female *Colletes halophilus* were not attractive for patrolling males of *Colletes hederae* (L. Calle, pers. comm. 2005).

Conclusions

Genetic differences, distribution patterns, phenology and flower specialisation show that *Colletes halophilus*, *Colletes hederae* and *Colletes succinctus* are separate species. The small number of distinguishing substitutions indicates that the taxa do not belong to a single gene pool, that they are close relatives, and that they have diverged very recently, but the molecular data does not allow the reconstruction of a phylogeny. However, relations of bee distribution patterns with those of their host plants and cuckoo bees imply a phylogenetic scenario consistent with the available molecular data. The congruence of distribution patterns between *Colletes succinctus* and its host plants and the cuckoo bee *Epeolus cruciger* can be hypothesised to represent a phylogenetically older system in which the species have reached a high degree of adaptation. In contrast, *Colletes hederae* and especially *Colletes halophilus* inhabit only a fraction of the respective distribution area of their host plants and seem to have insufficiently adapted cuckoo bees, which supports the hypothesis of a more recent origin. Thus, both species could be descendants of *Colletes succinctus*. This view is supported by the fact that in some populations and under certain conditions females of *Colletes succinctus* tend to collect pollen from *Hedera* or Asteraceae, the respective pollen sources of *Colletes hederae* and *Colletes halophilus*, instead of visiting its original host plants, *Calluna*

vulgaris and *Erica* spp. A switch to a new host plant is known as a factor promoting speciation (Moldenke 1979; Feder et al. 1988) and might be responsible for evolutionary processes in the *Colletes succinctus* group, too. Speciation processes of pollen-specialist (oligolectic) bees are generally little understood (Thorp 1969). Further investigations are required to reliably reconstruct the phylogeny of the *Colletes succinctus* group, to trace its evolutionary history and the ecological processes leading to the formation of new species. This would open up new perspectives for a better understanding of speciation of oligolectic bees in general.

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