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Identity of *Schizaphis* species (Hemiptera: Aphididae) in the United Kingdom: are they a threat to crops?

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Abstract

The greenbug, *Schizaphis graminum* (Rondani), is a major pest of cereals in some parts of the world and is of particular concern because it can be resistant to some insecticides and overcome the resistance of crops. In the UK, it has never been found on crops, but two rather little-known and closely-related species (*Schizaphis holci* and *Schizaphis agrostis*) are associated with the wild grasses, *Holcus lanatus* and *Agrostis stolonifera*. Since 1987, winged (alate) aphids morphologically resembling the greenbug have been found in increasing numbers in 12.2 m high suction-trap samples of the Rothamsted Insect Survey (RIS); hence, studies were undertaken to establish their identity. Clones (= asexual lineages) established from populations collected from *H. lanatus* in southern England showed strong preference for *Holcus* over *Agrostis* and *Hordeum* in laboratory tests and produced sexual morphs when transferred to short-day conditions, the males being apterous, as expected for *S. holci*. Multivariate morphometric comparisons of alatae caught in UK RIS suction traps in 2007 and 2011 with named specimens from museum collections, including *S. graminum* from many countries, indicated that the suction-trapped alatae were mostly *S. agrostis* and *S. holci*. Cytochrome *c* oxidase subunit I (COI) mtDNA obtained from 62 UK specimens from suction-traps had 95.4–100% sequence identity with US specimens of *S. graminum*. Two of the UK specimens had identical COI sequence to the US sorghum-adapted form of *S. graminum*, and these specimens also had 100% identity with a 640 bp fragment of nDNA CytC, indicating that this form of *S. graminum* may already be present in the UK. Present and future economic implications of these results are discussed.

Keywords: *Schizaphis agrostis*, *graminum*, *holci*, greenbug, biotype, genetic diversity, life history, morphometrics, suction traps

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Introduction

The greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), is a serious pest of small grain cereals, sorghum and turfgrasses in the USA (Hill, 1987; Blackman & Eastop, 2007). It causes direct damage through toxic secretions which produce yellow and brown lesions around the feeding sites as well as by transmitting a virus species (unassigned in the family Luteoviridae), which causes barley yellow dwarf and cereal yellow dwarf diseases (Lapierre & Signoret, 2004). The greenbug's pest status is exacerbated by resistance to organophosphate and carbamate insecticides (Sloderbeck *et al.*, 1991; Shufran *et al.*, 1996, 1997), and development of the ability to colonize and damage previously resistant cereal crops (Porter *et al.*, 1997).

The aphid is of Palaearctic origin and is recorded as a pest of sorghum in Russia (Radchenko & Lychagina, 2003) and wheat in Saudi Arabia (Alsuhaibani, 1996) and Pakistan (Aslam *et al.*, 2004). It has also sporadically caused severe problems in the Kenya highlands (Walker, 1954). In Europe, it is present, but is not a major cereal pest, in Greece (Tsitsipis *et al.*, 2007), Serbia (Tomanovic *et al.*, 2008), Spain (Juan Nieto Nafría, personal communication) and Italy (Sebastiano Barbagallo, personal communication).

In the UK, *S. graminum* had, until the present study, never been recorded. However, other *Schizaphis* species had been found but were considered to be rare, with little known of their taxonomy and biology (Stroyan, 1984); the scientific literature is based on very few specimens. The taxonomy of *Schizaphis* is uncertain and mainly based on host plant data as morphological identification is very difficult. There are two *Schizaphis* species found in the UK, *Schizaphis agrostis* Hille Ris Lambers and *Schizaphis holci* Hille Ris Lambers that are morphologically very similar to *S. graminum* and have even been classed as subspecies by Stroyan (1984). They are, even so, considered to be host-specific on *Agrostis* species (bent grasses) and *H. lanatus* L. (Yorkshire fog grass), respectively, and are not known to attack cereals (Hill, 1987). They are both thought to be monecious and holocyclic and limited information available about them suggests that the males of *S. agrostis* are winged, while those of *S. holci* are wingless (Hille Ris Lambers, 1947; Stroyan, 1984; Blackman & Eastop, 2006). *S. graminum* is monoecious and holocyclic with winged males in cold temperate climates but anholocyclic where winters are warm enough for survival of the mobile stages (Blackman & Eastop, 2006). Aphids flying throughout the UK have been monitored using 12.2 m tall suction-traps (Macaulay *et al.*, 1988) of the Rothamsted Insect Survey, RIS, since 1965 (Taylor, 1986; Harrington &Woiwod, 2007). Until 1987, there were very few records of *Schizaphis* spp. in these samples, after which numbers increased significantly, especially after the year 2000.

Even though no outbreaks of any *Schizaphis* species have so far been recorded from any crops in the UK, it is clearly important to investigate taxonomic relationships to the US greenbug and to draw attention to the increase in abundance of what has been considered a rare aphid genus in the UK, in order to assess the possibility that they may sooner or later become crop pests in the UK. Morphometric analyses and mitochondrial DNA gene and nuclear DNA intron sequence analyses were used to clarify the identity of individuals of UK *Schizaphis*, together with experimentation on host preference, life cycle and life history.

Materials and methods

Aphids studied

Insects were collected using 12.2 m high RIS suction-traps at 14 sites around the UK (fig. 1). The traps sample air at $0.75\,m^3\,s^{-1}$ and run continuously (Macaulay *et al.*, 1988). Aphid samples were taken daily during the 'aphid season' – from early April to mid-November – and weekly at other times. The trend over time for the mean flight date at the Rothamsted trap (years 1998–2010) was examined using regression analysis with year as the explanatory variable.

For the morphometric analyses described below, the aphids from suction traps were compared with specimens of *S. agrostis*, *S. graminum* and *S. holci* from the collection of the Natural History Museum, London (NHML).

Aphids were also collected from *H. lanatus* from the fields around Rothamsted Research, Hertfordshire, UK, and from a site near Luton, Bedfordshire, UK. Two clones (=asexual lineages, although their genetic fidelity was not tested using high resolution molecular markers *sensu* Loxdale *et al.*, 2013) were established and used for experimental work in the study. They originated from a single asexual (parthenogenetic) female collected from *H. lanatus* at Rothamsted on 2 June 2010 (Clone A; denoted UK_AX1 and UK_AX2 in the molecular analyses) and from a single asexual female collected from *H. lanatus* at Luton on 25 June 2010 (Clone L1; denoted UK_L1X1 and UK_L1X2 in the molecular analyses). Both lineages were reared on *H. lanatus* at 18°C and a photoperiod of 16:8 (L:D) hours. No aphids were found locally on *Agrostis* spp.

Host choice

Clones A and L1 were tested for their preference for three potential hosts: (i) *H. lanatus* (the host from which the aphid was collected in the field); (ii) *Agrostis stolonifera* (creeping bent, a potential host for *S. agrostis*); and (iii) *Hordeum vulgare* (barley, cv. 'Saffron').

The three plant species were sown in the same pot (12.7 cm diameter), near the edge, at equal distances from the centre and between themselves. *H. lanatus* and *A. stolonifera* plants were two weeks old and barley was one week old when the experiment was performed. Even then, barley was a larger plant compared with the other two. In order to equalize the quantity of plant material, four to five plants of *H. lanatus* and *A. stolonifera* were used, but only one barley plant. A piece of filter paper (12.5 cm diameter) was used to cover the soil and the aphids (ten adult apterae of one clone per pot) were released at the centre of the pot. Eight replicates were done for Clone A and seven replicates for Clone L1. They were then scored after 24 h, 48 h and 7 days. Numbers of adults and nymphs were recorded on each host and the nymphs were removed after every scoring. Each pot was kept isolated inside a perforated plastic bag, and all the bagged pots were kept at 18°C, 16:8 (L:D) hours.

Rate of increase

Twenty adult aphids of each clone (A and L1) were placed individually on plants (either *H. lanatus* or barley) and left to reproduce. One of their first-born progeny was allowed to reach adulthood and reproduce. Plants were covered individually with a plastic cylinder with openings covered with fine

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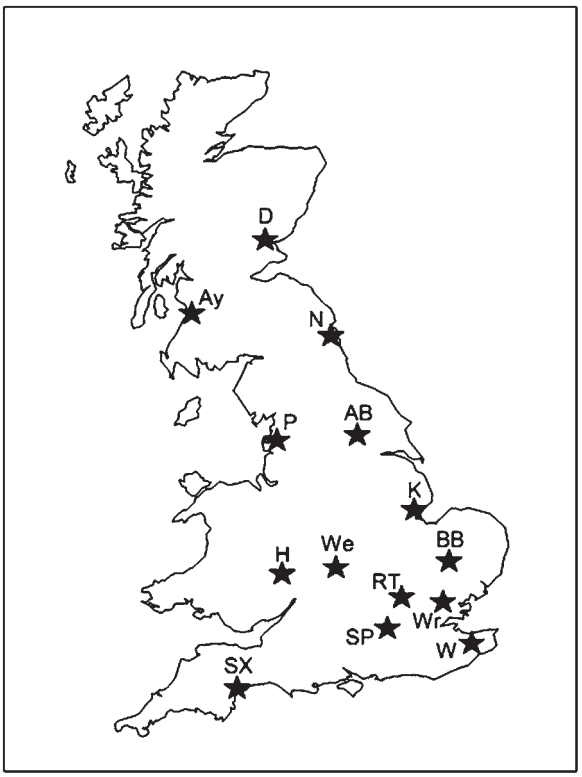

★ : indicate suction trap sites which are:

D	Dundee	We	Wellesbourne
Ay	Ayr	H	Hereford
N	Newcastle	RT	Rothamsted Tower
AB	Askham Bryan	Wr	Writtle
P	Preston	SP	Silwood Park
K	Kirton	W	Wye
BB	Broom's Barn	SX	Starcross

Fig. 1. The network of suction traps across the UK.

mesh for ventilation. The progeny were counted daily and removed with a fine paintbrush. The intrinsic rate of increase r_m was calculated for each aphid using the formula of Wyatt & White (1977).

Life cycle category

For each clone (A and L1), ten fourth-instar nymphs (generation G0) were transferred from the long-day cultures (18°C, 16:8 [L:D] hours) to short-day conditions (14°C, 10:14 [L:D] hours) individually on *H. lanatus* leaves in ampoules (Austin *et al.*, 1991). Their five first-born progeny (G1) were isolated and allowed to reach adult stage and reproduce. At this point, five late-born G1 were isolated from the G0 parents and reared to adulthood and their morph (winged or wingless asexuals, winged males and wingless sexual females, i.e., oviparae) assessed. The five first-born progeny of the G1 (i.e., the G2) were allowed to reach adulthood and their morph assessed. At the same time as the first-born G2 reached adulthood, five late-born G2 were isolated and reared to adulthood and their morph assessed. This regime was used to discern whether or not the clones produced oviparae (sexual females) and males (Mittler & Gorder, 1991).

Sexual morphs were identified at the adult stage, males from their genitalia, oviparae from their characteristic swollen hind tibiae with scent plaques. The *H. lanatus* leaves in the ampoules were changed as needed throughout the study.

Morphometric analyses

Aphids were mounted on conventional glass slides using the method of Martin (1983). Measurements were made of 238 alate specimens: 68 from the NHML collection (table 1) and 170 collected in 2007 and 2011 from ten suction-traps in the UK. These years were selected because of the especially high abundance of *Schizaphis* spp. collected. Eleven morphological characters, generally used in taxonomy of Aphidinae, and of the *S. graminum* group in particular (Stroyan, 1984; Fargo *et al.*, 1986; Heie, 1986; Inayatullah *et al.*, 1987; Rubin-de-Celis *et al.*, 1997; Blackman & Eastop, 2006) were measured for each specimen. These characters with their abbreviations are given in table 2.

The length of the longest hair on abdominal tergite VIII (HL VIII) was included in the measured character list as it is known to be a useful character for separation of wingless (apterous) females of *S. agrostis* from *S. holci*, but it was not used in the multivariate analyses other than as an independent variable to justify groupings. The measurements were done according to Ilharco & van Harten (1987) and Blackman & Eastop (2006) using a Zeiss Axioskop microscope fitted with a microscope camera and the program InSight ver. 1.14.4 (DeltaPix, Maalov, Denmark). The mean value and its standard deviation (SD) for each morphological character were calculated.

Patterns of morphometric variation were analyzed using two multivariate statistical approaches (Tabachnick & Fidell, 2006) with ten variables, excluding HL VIII, which was used as an independent character for separation of suction-trapped *Schizaphis* spp. in the canonical discriminant analysis (CDA). Principal component analysis (PCA) assesses components of the total of variation among all specimens by calculating a linear combination of the variables that explains the maximum amount of total variation, and then iteratively calculates new combinations to explain any residual variation. This procedure does not assume any *a priori* groupings. CDA operates on the mean values for groups defined prior to analysis, effectively providing linear combinations of variables that best summarize differences between classes. PCA and CDA were based on the correlation matrix of the coefficients (Tabachnick & Fidell, 2006; Abdi & Williams, 2010). Using CDA, the individuals were divided into six groups: (i) *S. holci* from *H. lanatus*; (ii) *S. agrostis* from *Agrostis* and *Poa*; (iii) *S. graminum* from hosts in countries outside the UK; (iv) 114 suction-trapped specimens from the UK identified as *S. agrostis* (HL VIII up to 0.021 mm, see table 9); (v) 36 identified as *S. holci* (HL VIII 0.026–0.048 mm); and (vi) 20 individuals with intermediate values of this character (HL VIII 0.022–0.025 mm). Means of each variable were compared using a one-way analysis of variance (ANOVA). *F*-values and Wilks' Lambda were computed for each variable to determine the overall between-group differentiation. The analyses were performed using the software packages GenStat ver. 12 (Payne *et al.*, 2009) and Past ver. 2.16 (Hammer *et al.*, 2001).

One male of *S. agrostis* (labeled as a cotype, an old term for syntype – a member of a type series in which no holotype or lectotype has been designated) collected on *Agrostis alba*,

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Table 1. Collection information for *Schizaphis* species samples used in morphometric analysis.

Species	Country	Place	Host plant	Data	Number
S. graminum	Angola	Sahama, Caconda	*Triticum*	24.07.1964	4
		Sanguete, Caconda		22.07.1964	4
	Brazil	Pelotas	*H. vulgare*	18.05.1968	1
	Egypt	Cairo	*Cynodon dactylon*	18.03.1962	5
	Eritrea	Asmara	*C. dactylon*	29.05.1950	6
	Georgia	–	*Sorghum*	–	1
		Archiloskalo, Dedoplistskaro	*Triticum aestivum*	04.05.1965	1
		Shiraki, Dedoplistskaro		01.04.1966	1
		Dedoplistskaro		24.03.1960	1
	India	Puna (formerly Poona)	*T. aestivum* (=*Triticum vulgare*)	05.1961	1
	Kenya	Njoro	*T. aestivum* & *H. vulgare*	11.08.1962	2
	Mexico	Irapuato	*Fragaria* ?	10.04.1982	2
	Pakistan	Quetta	*Sorghum sudanense*	18.09.1970	1
	Romania	Studina	*Zea mays*	20.06.1958	1
	Sudan	Wad Madani	*T. aestivum*	03.1963	1
	USA	Riverside, California	*H. vulgare*	19.08.1968	2
		Guymon, Oklahoma	*Sorghum* in culture	11.1978	2
		Dallas, Texas		11.1978	2
	Zimbabwe (South Rhodesia)	Harare (formerly Salisbury)	*T. aestivum*	16.09.1958	2
	Yugoslavia	–	*T. aestivum* (=*Triticum sativum*)	23.06.1962	9
	Locality is unclear on slide	–	*T. aestivum*	–	1
S. agrostis	The Netherlands	Bennekom	*Agrostis canina*	23.06.1944	2
		Wageningen-Hoog	*Poa annua*	19.06.1938	3
				08.1938	1
S. holci	The UK	Harpenden	*H. lanatus*	25.03.2010	1
				09.06.2010	4
				10.06.2010	4
				14.06.2010	3

Table 2. Morphological characters used and their abbreviations.

Morphological character	Abbreviation
Length of processus terminalis	PT
Length of the base of sixth antennal segment	ANTVIB
Length of third antennal segment	ANTIII
Basal diameter of third antennal segment	BDANTIII
Total length of rostrum	ROSTRUM
Length of fourth + fifth rostral segments	URS
Length of hind femur	HFEM
Length of hind tibia	HTIB
Length of second segment of hind tarsus	HTII
Length of siphunculi	SIPH
Length of hairs on eighth abdominal tergite	HL VIII
Length of cauda	CAUDA

15 males from the two clones of *S. holci* reared in laboratory conditions at Rothamsted, and 41 suction-trapped males of unidentified *Schizaphis* species were compared by measuring ultimate rostral segment (URS) and HTII and preparing a bivariate plot.

DNA sequence analyses

DNA sequences were obtained from 61 alate specimens of *Schizaphis* collected in the UK in 2009 and 2010, 50 from suction traps and 11 from *Holcus* (table 3). Genomic DNA was extracted from single aphids using the *prep*GEM™ Insect DNA extraction kit (ZyGEM Corp. Ltd, Hamilton, New Zealand) according to the manufacturer's instructions, except the volume of the extraction mix was reduced by 50% (20 µl). Aphids stored in 95% ethyl alcohol were rinsed twice with 100 µl sterile distilled, deionized water before extraction. A 640 bp fragment of the mtDNA cytochrome *c* oxidase subunit I (COI) gene was polymerase chain reaction (PCR) amplified from all aphids using the primers LepF (5′-ATTCAACCAAT-CATAAAGATATTGG-3′) and LepR (5′-TAAACTTCTG-GATGTCCAAAAAATCA-3′) (Hajibabaei *et al.*, 2006). A 640 bp fragment of the nDNA cytochrome *c* (CytC) gene was PCR amplified from four aphids (based on their close relatedness to US biotypes) using the primers cytC-C-5′ (5′-AAGTGTGCYCARTGCCACAC-3′) and cytC-B-3′ (5′-CAT-CTTGGTGCCGGGGATGTATTTCTT-3′) (Palumbi, 1996). This product contained intron regions which were used in phylogenetic analyses. The reaction conditions were: 25 µl volume; 10 ng template DNA; 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 0.2 mM dNTPs; 2.5 mM $MgCl_2$; 20 pmol of each primer; and 1.5 U Go*Taq*DNA polymerase (Promega, Madison, WI, USA). An MJ PTC-100 Thermal Controller was used with the following program steps: (i) 96°C 3 min (denaturation); (ii) 94°C 30 s; (iii) 50°C 30 s (annealing); (iv) 72°C 1 min (extension); (v) cycle to step 2, 34 times; (vi) 72°C 5 min; (vii) 4°C hold. The presence of PCR regions of correct size was determined using standard 1.5% agarose gel electrophoresis (Sambrook *et al.*, 1989).

PCR products were directly purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). DNA sequences for both positive and negative strands were obtained using BigDye™ (Applied Biosystems, Foster City, CA, USA) terminated reactions with an ABI 3700 DNA Analyzer at the Recombinant DNA/Protein Resource Facility, Oklahoma State University, Stillwater, Oklahoma, USA. Each DNA region was subjected to 4–5× coverage and nucleotide sequences were assembled with SeqMan in the LaserGene™ version 8.0 (DNASTAR, Madison, WI, USA) package.

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Table 3. UK samples of *Schizaphis* used in DNA sequence analyses with COI accession numbers for DNA sequences submitted to GenBank.

Sample ID	Location	Collection date	Host	COI accession No.
AB3	Askham Bryan	16 June 2009	Suction trap	JN383533
BB1	Broom's Barn	01 May 2009	Suction trap	JN383532
BB2	Broom's Barn	02 May 2009	Suction trap	JN383549
BB5	Broom's Barn	06 May 2009	Suction trap	JN383590
BB7	Broom's Barn	28 May 2009	Suction trap	JN383564
BB8	Broom's Barn	31 May 2009	Suction trap	JN383563
BB9	Broom's Barn	13 June 2009	Suction trap	JN383562
BB12	Broom's Barn	13 July 2009	Suction trap	JN383561
H1	Hereford	25 May 2009	Suction trap	JN383547
H2	Hereford	27 May 2009	Suction trap	JN383545
H4	Hereford	31 May 2009	Suction trap	JN383591
H5	Hereford	31 May 2009	Suction trap	JN383543
K1	Kirton	29 April 2009	Suction trap	JN383531
K3	Kirton	22 June 2009	Suction trap	JN383541
K4	Kirton	22 June 2009	Suction trap	JN383539
UK21	Luton	25 June 2010	*H. lanatus*	JN383574
UK23	Luton	25 June 2010	*H. lanatus*	JN383572
UK_L1X1	Luton	25 June 2010	*H. lanatus*	JN383566
UK_L1X2	Luton	25 June 2010	*H. lanatus*	JN383565
RT1	Rothamsted	10 May 2009	Suction trap	JN383560
RT2	Rothamsted	15 May 2009	Suction trap	JN383559
RT7	Rothamsted	14 June 2009	Suction trap	JN383558
RT9	Rothamsted	27 June 2009	Suction trap	JN383557
RT10	Rothamsted	11 July 2009	Suction trap	JN383556
RT11	Rothamsted	23 May 2009	Suction trap	JN383555
RT12	Rothamsted	24 May 2009	Suction trap	JN383554
RT13	Rothamsted	28 May 2009	Suction trap	JN383553
RT14	Rothamsted	01 June 2009	Suction trap	JN383552
RT15	Rothamsted	13 June 2009	Suction trap	JN383551
UK2	Rothamsted	20 May 2010	Suction trap	JN383589
UK3	Rothamsted	21 May 2010	Suction trap	JN383588
UK4	Rothamsted	22 May 2010	Suction trap	JN383587
UK5	Rothamsted	23 May 2010	Suction trap	JN383586
UK8	Rothamsted	31 May 2010	Suction trap	JN383585
UK25	Rothamsted	02 June 2010	*H. lanatus*	JN383570
UK_AX1	Rothamsted	02 June 2010	*H. lanatus*	JN383568
UK_AX2	Rothamsted	02 June 2010	*H. lanatus*	JN383567
UK20	Rothamsted	03 June 2010	*H. lanatus*	JN383575
UK22	Rothamsted	03 June 2010	*H. lanatus*	JN383573
UK10	Rothamsted	04 June 2010	Suction trap	JN383584
UK11	Rothamsted	04 June 2010	Suction trap	JN383583
UK12	Rothamsted	05 June 2010	Suction trap	JN383582
UK13	Rothamsted	06 June 2010	Suction trap	JN383581
UK14	Rothamsted	09 June 2010	Suction trap	JN383580
UK26	Rothamsted	10 June 2010	*H. lanatus*	JN383569
UK15	Rothamsted	11 June 2010	Suction trap	JN383579
UK24	Rothamsted	15 June 2010	*H. lanatus*	JN383571
UK16	Rothamsted	17 June 2010	Suction trap	JN383578
UK17	Rothamsted	20 June 2010	Suction trap	JN383577
UK18	Rothamsted	25 June2010	Suction trap	JN383576
Sp1	Silwood Park	10 May 2009	Suction trap	JN383550
Sp2	Silwood Park	14 May 2009	Suction trap	JN383548
W1	Wye	05 May 2009	Suction trap	JN383546
We2	Wellesbourne	25 May 2009	Suction trap	JN383537
We4	Wellesbourne	29 June 2009	Suction trap	JN383535
We5	Wellesbourne	09 July 2009	Suction trap	JN383544
Wr2	Writtle	14 May 2009	Suction trap	JN383542
Wr3	Writtle	24 May 2009	Suction trap	JN383540
Wr5	Writtle	31 May 2009	Suction trap	JN383538
Wr7	Writtle	12 June. 2009	Suction trap	JN383536
Wr9	Writtle	30 June 2009	Suction trap	JN383534

Sequences were then aligned by the ClustalW method (Thompson *et al.*, 1994) using MegAlign in the LaserGene™ software package. Default alignment parameters were used; gap penalty 15.0, gap length penalty 6.66, delay divergent sequences 30% and DNA transition weight 0.5. Phylogenetic analyses were conducted using the MEGA5 statistical software package (Tamura *et al.*, 2011). Maximum likelihood (ML) method with 1000 bootstraps was used based on the Tamura &

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Table 4. GenBank accession numbers of DNA sequences of specimens of US *S. graminum* biotypes used in analyses (Shufran, 2011; Shufran & Puterka, 2011).

Biotype	COI accession no.	CytC accession no.
B	HQ392572	JF719756
B-OK	HQ392581	JF719752
C	HQ392573	JF719744
E	HQ392575	JF719745
E-OK	HQ392579	JF719753
F	HQ392576	JF719746
G	HQ392577	JF719747
H	HQ392578	JF719748
I	HQ392582	JF719749
J	HQ392583	JF719757
K	HQ392584	JF719750
NY	HQ392585	JF719751
Paspalum vaginatum (FL or P)	HQ392586	JF719755
Unknown (?)-OK	HQ392580	JF719754

Nei (1993) model, with uniform substitution rates among sites, all sites (gaps and/or missing data) used, and the ML heuristic method Nearest-Neighbour-Interchange. Included in the analyses were COI and CytC sequences from *S. graminum* collected in the USA (Shufran *et al.*, 2000; Shufran, 2011; Shufran & Puterka, 2011) (table 4). DNA sequences were submitted to GenBank with accession numbers JN383531–JN383591 (COI) (table 3) and JN383592–JN383595 (CytC).

Results

Suction traps and field collections

Table 5 shows the numbers of female and male aphids identified as *Schizaphis* spp. caught in the UK RIS suction-traps for the years 1987–2010. From the beginning of the operation of the trap network (1964) until 1987 only nine such individuals were found. Since then numbers have increased dramatically. Apart from the most northern traps in Dundee and Ayr, the aphids were caught throughout the UK in all the remaining 12 traps but numbers were much higher in the south. Fig. 2 shows the total caught in the Rothamsted suction trap each week, averaged for the years 1987–2010. Peak flight occurred in late May and the males appeared in late September. The mean flight date at the Rothamsted trap has become earlier in recent years (fig. 3, $F_{1,11} = 6.85$, $P < 0.05$; for years 1998–2010).

The fields around Rothamsted were searched in June and July 2010 and 2011. One location near Luton was also searched in June 2010. Aphids of the genus *Schizaphis* were only found on *H. lanatus* and not on *Agrostis* nor any other grass. No ant attendance was observed.

Host choice

The aphids showed a clear preference for the host on which they were found in the field and reared on in the laboratory (*H. lanatus*) (table 6). Thirty minutes after their release, aphids moved towards or onto *H. lanatus* (A. Kati, personal observation). For Clone A, only one adult was found on *A. stolonifera* and it produced five nymphs during one week. Only two adults were found on barley and they produced 16 nymphs during one week. For Clone L1, only one adult was found on *A. stolonifera* and produced two nymphs during one week. Only two adults were found on barley and they produced eight nymphs during one week.

An attempt to culture the clones on barley in a no-choice experiment failed. A very small number of aphids survived and produced very few progeny but the population soon died out. Aphid feeding caused chlorosis both on *H. lanatus* and barley.

Rate of increase

The average total number of progeny produced by each adult was 36.8 and 37.1, the mean intrinsic rate of increase for clones A and L1 on *H. lanatus* was 0.223 and 0.215, respectively.

On barley, only two out of 20 Clone A adults reproduced and their first-born progeny reached adulthood and produced seven and eight nymphs, respectively. A third one reproduced, but its first-born did not. The rest produced no progeny and were either not found after a few days or found dead. Two out of 20 Clone L1 adults reproduced and their first-born progeny reached adulthood and produced seven and 23 nymphs, respectively. The rest produced no progeny and were either not found after a few days or found dead. Owing to the very small number of aphids surviving and reproducing on barley, the mean intrinsic rate of increase was not calculated.

Life cycle category

Both Clones A and L1 produced males and oviparae. The first-born G1 were virginoparae and the late-born G1 virginoparae and males. The first-born G2 were oviparae, whereas the late-born G2 were oviparae and males (fig. 4). Most males possessed narrow sclerotized thoraxes with no wing buds or with rudimentary wing buds or deformed wings. No fully winged males were produced.

Morphometric analyses

Contributions of the ten variables to the first two principal components (PCs), accounting for 82% of total variation, are given in table 7. PC 1 (74% of total variation) reflects generalized body size (contributions by all variables are positive and of approximately the same magnitude). The main contributors to PC 2 (8% of total variation) were URS, ANTVIB and BDANTIII as these variables had large positive or large negative coefficients. A plot of PC 1 against PC 2 shows a clear separation between *S. graminum* and all remaining *Schizaphis* samples (host plant-collected *S. agrostis*, and *S. holci* and all *Schizaphis* from suction-trap samples) (fig. 5; table 7). The *Schizaphis* individuals from suction-traps formed two loose clusters, with individuals of host plant-collected *S. agrostis* and *S. holci* located within each of these clusters.

Using CDA the individuals were divided into six groups. PCA results provided a sufficient basis for allocating alatae collected from host plants to three of these groups; *S. holci* from *Holcus*, *S. agrostis* from *Agrostis* and *Poa*, and *S. graminum* from hosts in countries outside the UK. Alatae trapped in the UK were allocated to three groups on the basis of measurements of HL VIII (see the Materials and methods section). The first and second canonical variates (CVs) explained 72% and 25% of the total variation, respectively (table 7). The variables contributing most to CV 1 were BDANTIII and HTII (large positive coefficient) and URS and ANTVIB (large negative coefficient). The variables contributing most to CV 2 were HTII (positive)

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Table 5. Numbers of female and male aphids identified as *Schizaphis* spp. caught in the UK suction traps for the years 1987–2010. ni, not yet identified; no, trap not operating; dm, data missing due to trap not operating for part of the year or malfunctioning, for trap names see fig. 1.

	D		Ay		N		AB		P		K		BB		We		H		RT		Wr		SP		W		SX		Year Totals		
Year	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀ and ♂
1987	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	1	0	0	0	0	0	3	0	0	0	0	0	4	0	4
1988	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	8	0	0	0	0	0	8	0	8
1989	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	0
1990	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	1	0	1	0	1
1991	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	dm	dm	no	no	0	0	0	0	0	0	0
1992	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	0
1993	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	2	0	0	0	no	no	2	0	0	0	4	0	4
1994	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	0
1995	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	0
1996	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	no	no	0	0	0	0	0	0	0
1997	0	0	0	0	0	0	no	no	1	0	0	0	1	0	no	no	0	0	0	0	1	0	no	no	1	0	4	0	8	0	8
1998	0	0	0	0	1	0	no	no	0	0	0	0	2	0	no	no	0	0	4	0	5	0	no	no	4	0	1	0	17	0	17
1999	0	0	0	0	0	0	no	no	0	0	0	0	2	0	no	no	0	0	5	0	5	0	no	no	5	0	12	0	29	0	29
2000	0	0	0	0	0	0	0	0	0	0	1	0	12	0	no	no	1	0	2	0	7	1	10	0	17	0	1	0	51	1	52
2001	0	0	0	0	0	0	0	0	0	0	1	0	7	1	no	no	0	0	4	0	10	1	0	2	3	0	4	0	29	4	33
2002	0	0	ni	ni	0	0	0	0	0	0	16	0	7	3	no	no	1	0	14	2	8	1	61	16	7	0	dm	dm	114	22	136
2003	0	0	0	0	0	0	2	0	0	0	0	0	60	0	no	no	dm	dm	17	0	26	0	10	3	28	0	15	2	158	5	163
2004	0	0	0	0	1	0	0	0	2	0	7	0	36	0	8	0	5	0	27	0	46	0	21	1	31	1	10	0	194	2	196
2005	0	0	0	0	1	0	3	0	0	0	6	0	9	0	8	0	0	1	16	1	11	2	11	3	26	dm	11	0	102	7	109
2006	ni	ni	ni	ni	0	0	1	2	0	0	5	0	37	4	11	6	10	dm	38	40	32	6	31	14	30	4	111	16	306	92	398
2007	ni	ni	ni	ni	0	0	13	0	0	0	10	2	69	9	36	3	3	2	154	16	85	15	284	8	100	10	118	2	872	67	939
2008	ni	ni	ni	ni	2	0	3	0	0	0	5	0	15	0	4	0	1	0	8	2	23	0	11	6	13	0	11	0	96	8	104
2009	ni	ni	ni	ni	ni	ni	8	0	1	0	27	0	169	0	ni	ni	10	0	107	10	100	0	ni	ni	ni	ni	ni	ni	422	10	432
2010	ni	ni	ni	ni	0	0	12	0	0	0	4	0	14	0	ni	ni	5	0	37	0	47	0	ni	ni	20	0	ni	ni	139	0	139
Trap total	0	0	0	0	5	0	42	2	4	0	82	2	440	17	67	9	37	3	435	71	406	26	450	53	287	15	299	20	2554	218	2772

Table 6. Mean (±SE) of clones A and L1 *S. holci* adults present on each host and nymphs produced per adult on each of the three host plants after 24 h, 48 h and 1 week. Means followed by the same letter within the same section in a column are not significantly different ($P > 0.05$; paired student's t test).

	Host	Adults/host		Nymphs/adult	
		clone L1	clone A	clone L1	clone A
After 24 h	*H. lanatus*	7 ± 0.7 a	7.88 ± 0.5 a	2.5 ± 0.2 a	2.15 ± 0.2 a
	A. stolonifera	0.14 ± 0.1 b	0.13 ± 0.1 b	0 ± 0 b	0.13 ± 0.1 b
	Barley	0.29 ± 0.2 b	0.25 ± 0.2 b	0.14 ± 0.1 b	0.25 ± 0.2 b
After 48 h	*H. lanatus*	6.57 ± 0.4 a	7.25 ± 0.6 a	3.15 ± 0.3 a	2.23 ± 0.2 a
	A. stolonifera	0 ± 0 b	0.13 ± 0.1 b	0 ± 0 b	0.13 ± 0.1 b
	Barley	0 ± 0 b	0.25 ± 0.2 b	0 ± 0 b	0.13 ± 0.1 b
After 1 week	*H. lanatus*	5.43 ± 0.9 a	6.75 ± 0.6 a	13.4 ± 2.6 a	7.09 ± 0.6 a
	A. stolonifera	0.14 ± 0.1 b	0 ± 0 b	0.14 ± 0.1 b	0 ± 0 b
	Barley	0 ± 0 b	0.25 ± 0.2 b	0 ± 0 b	1 ± 0.9 b

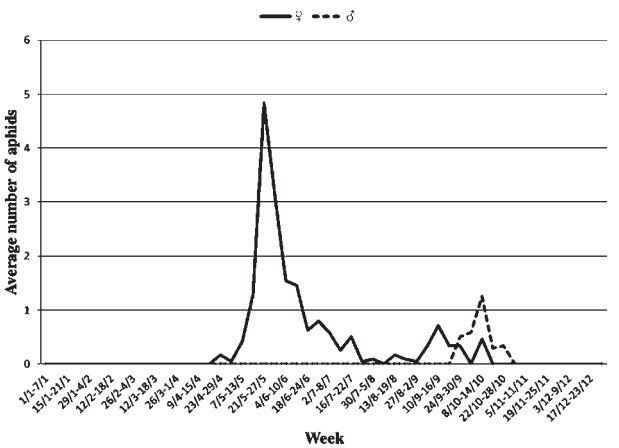

Fig. 2. Phenology curve showing the total number of female and male aphids identified as *Schizaphis* spp. caught in the Rothamsted suction trap averaged for every week for the years 1987–2010.

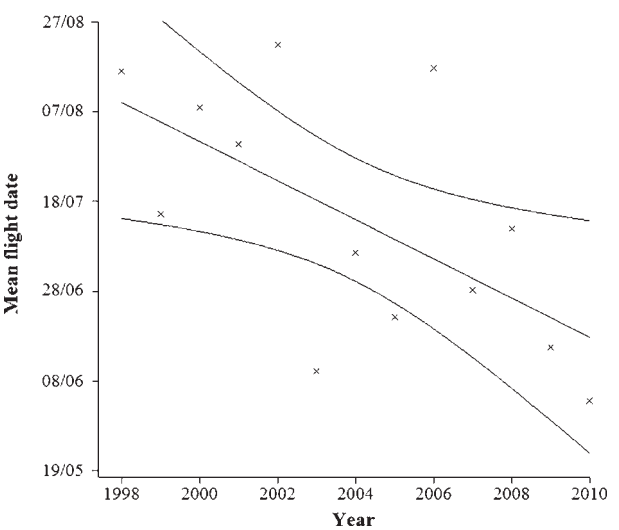

Fig. 3. Mean flight date of female and male aphids identified as *Schizaphis* spp. caught in the Rothamsted suction trap for the years 1998–2010.

Table 7. Proportion of contribution and variable coefficients of first two eigenvectors (PCs) for PCA and total sample standardized canonical coefficients for CDA in alatae of the *Schizaphis* spp. ($n = 238$). Variable names are defined in table 2.

Variable	PC 1	PC 2	CV 1	CV 2
PT	0.2794	0.2958	0.0058	0.0001
ANTVIB	0.2924	0.3858	−0.0427	−0.0298
ANTIII	0.3328	0.1439	−0.0111	0.0039
BDANTIII	0.2669	−0.6586	0.1136	0.0537
URS	0.2964	0.4565	−0.0567	−0.2659
HFEM	0.3456	−0.1494	−0.0004	0.0084
HTIB	0.3584	−0.0091	0.0147	−0.0013
HTII	0.3377	−0.2303	0.1331	0.0837
SIPH	0.3265	−0.1369	0.0092	0.0025
CAUDA	0.3132	−0.0815	−0.0046	−0.0179
Proportion of total variation	74%	8%	72%	25%

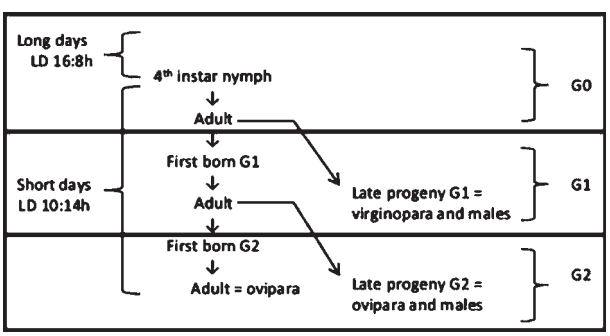

Fig. 4. The production of sexual morphs of *S. holci* under short-day conditions.

and URS (negative). Alatae of *S. graminum* were distinguished from all other remaining individuals by their much higher scores on CV 1 in combination with high scores on CV 2 (fig. 6). Trapped and host-collected *S. agrostis* had low scores on CV 1 and high scores on CV 2. Both trapped and host-collected *S. holci* had intermediate scores between *S. graminum* and *S. agrostis* on CV 1, while they had low scores on CV 2. Most of the trapped *Schizaphis* with intermediate values of the hair length character HL VIII grouped with trapped and host plant-collected *S. agrostis*. Group centroids of trapped

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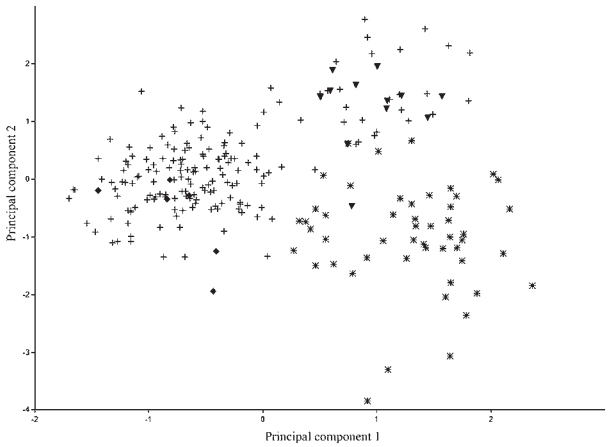

Fig. 5. PC ordination of 238 alate individuals of *Schizaphis* spp. based on the analysis of ten morphological variables, onto the first and second principal axes (Table 7). Symbols: *S. graminum* (*), *S. holci* from *Holcus* (▼), *S. agrostis* from *Agrostis* and *Poa* (♦), *Schizaphis* individuals from UK suction-traps (+).

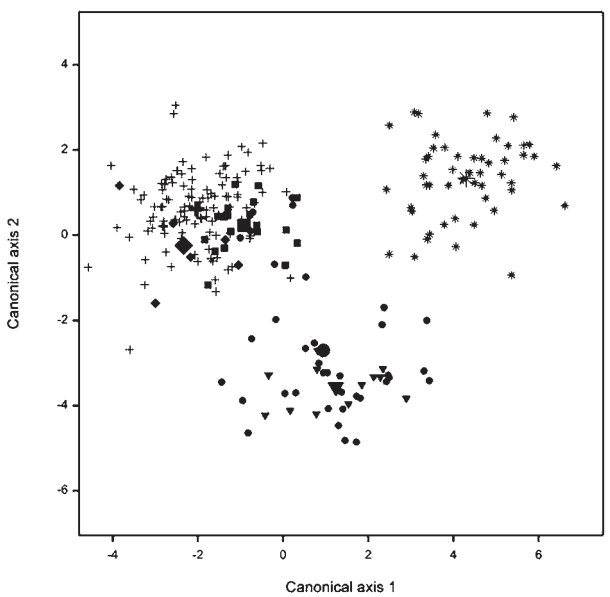

Fig. 6. CDA of 238 alate individuals of *Schizaphis* spp. based on the analysis of ten morphological variables; specimens projected onto the first and second canonical axes (Table 7). Symbols: *S. graminum* individuals and their group centroid (*), *S. holci* individuals from *Holcus* and their group centroid (▼), trapped *S. holci* individuals and their group centroid (●), trapped intermediate *Schizaphis* individuals and their group centroid (■), *S. agrostis* individuals from *Agrostis* and *Poa* and their group centroid (♦), trapped *S. agrostis* individuals and their group centroid (+).

and host-collected *S. agrostis* were close to each other and were clearly separated from group centroids of all remaining groups. The group centroid of trapped intermediate *Schizaphis* was close to group centroids of trapped and host-collected *S. agrostis*. It seems that most, if not all, of the individuals of the intermediate group belonged to *S. agrostis*, which was by far the commoner of the two *Schizaphis* species occurring in the suction-traps (table 8).

Table 8. Collection information for trapped *Schizaphis* spp. samples used in the study ($n = 170$).

N	Locality	*S. agrostis*	*S. holci*	Intermediate *Schizaphis*
1	Askham Bryan	7	0	3
2	Broom's Barn	17	1	1
3	Hereford	1	1	1
4	Kirton	2	3	1
5	Rothamsted	21	9	5
6	Silwood	12	2	0
7	Starcross	11	6	0
8	Wellesbourne	15	3	4
9	Writtle	16	6	1
10	Wye	12	5	4
Total		114	36	20

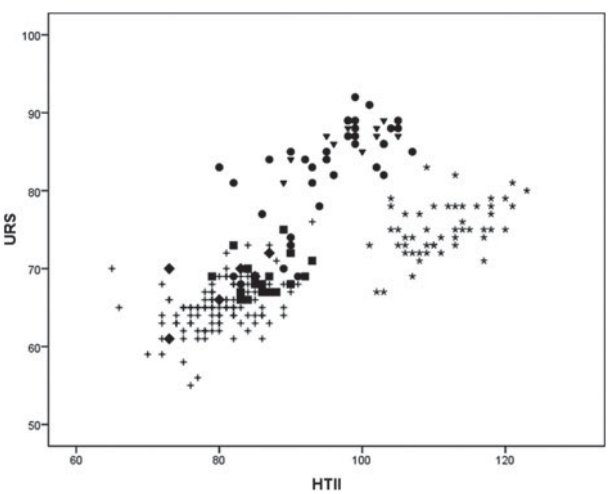

Fig. 7. Bivariate plot of the lengths in mm of URS versus second segment of hind tarsus (HTII) for alatae of *Schizaphis* spp. ($n = 238$). Symbols: *S. graminum* (*), *S. holci* from *Holcus* (▼), trapped *S. holci* (●), trapped intermediate *Schizaphis* individuals (■), *S. agrostis* from *Agrostis* and *Poa* (♦), trapped *S. agrostis* (+).

Examination of the raw data (table 9) showed that ranges of measurements of morphological variables mostly overlapped. Alatae of *S. agrostis* were smaller than either *S. graminum* or *S. holci*, as indicated by characters closely correlated with general size (ANT III, HTIB), and much of the difference between *S. agrostis* and the other two species is accounted for by this overall size difference.

The one-way ANOVA for each morphological character of the *S. graminum* group revealed the most influential variables for morphometric discrimination to be HFEM, HTIB, HTII and URS as they had smaller Wilks' Lambda and higher *F*-values (table 10), which indicate a greater difference between group means.

Best discrimination between *S. graminum* and *S. agrostis* + *S. holci* was with URS, HTIB and HTII (and HL VIII). A good two-character discrimination between alatae of *S. graminum* and host plant-collected and suction-trapped *S. agrostis* + *S. holci* was achieved in bivariate plots of URS versus HTII and URS versus HTIB (figs 7 and 8).

In the bivariate plot of URS versus HTII for males (fig. 9), suction-trapped (i.e., fully alate) *Schizaphis* individuals were

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Table 9. Measurements of morphological characters of alate females of the *Schizaphis* spp. (measurements given in mm). Variable names are defined in table 2.

Characters	*S. graminum* ($n=50$)		*S. agrostis* from *Agrostis* and *Poa* ($n=6$)		Trapped *S. agrostis* ($n=114$)		*S. holci* from *H. lanatus* ($n=12$)		Trapped *S. holci* ($n=36$)	
	Range	Means ± SD	Range	Means ± SD	Range	Means ± SD	Range	Means ± SD	Range	Means ± SD
PT	0.298–0.482	0.400 ± 0.046	0.289–0.347	0.316 ± 0.021	0.245–0.381	0.318 ± 0.029	0.216–0.433	0.378 ± 0.055	0.255–0.446	0.386 ± 0.042
ANTVIB	0.086–0.138	0.110 ± 0.011	0.079–0.098	0.086 ± 0.006	0.071–0.114	0.094 ± 0.009	0.111–0.143	0.121 ± 0.008	0.089–0.150	0.114 ± 0.014
ANTIII	0.238–0.377	0.304 ± 0.036	0.214–0.233	0.225 ± 0.008	0.182–0.292	0.239 ± 0.022	0.277–0.325	0.297 ± 0.015	0.239–0.354	0.296 ± 0.030
BDANTIII	0.020–0.032	0.025 ± 0.003	0.018–0.026	0.021 ± 0.003	0.014–0.024	0.020 ± 1.495	0.020–0.023	0.022 ± 0.001	0.018–0.025	0.021 ± 0.002
ROSTRUM	0.369–0.527	0.444 ± 0.030	0.342–0.432	0.392 ± 0.038	0.319–0.538	0.374 ± 0.029	0.419–0.491	0.453 ± 0.021	0.370–0.517	0.446 ± 0.035
URS	0.067–0.083	0.075 ± 0.003	0.061–0.072	0.068 ± 0.004	0.055–0.076	0.065 ± 0.003	0.081–0.089	0.085 ± 0.003	0.068–0.092	0.082 ± 0.007
HFEM	0.349–0.534	0.471 ± 0.40	0.310–0.383	0.350 ± 0.026	0.221–0.413	0.347 ± 0.027	0.368–0.456	0.410 ± 0.030	0.368–0.468	0.421 ± 0.026
HTIB	0.720–0.968	0.841 ± 0.064	0.516–0.639	0.586 ± 0.046	0.526–0.692	0.616 ± 0.039	0.728–0.882	0.787 ± 0.048	0.668–0.922	0.779 ± 0.063
HTII	0.101–0.123	0.111 ± 0.006	0.073–0.087	0.080 ± 0.006	0.065–0.093	0.080 ± 0.005	0.089–0.105	0.097 ± 0.005	0.079–0.107	0.094 ± 0.008
SIPH	0.169–0.244	0.208 ± 0.019	0.150–0.166	0.156 ± 0.006	0.122–0.185	0.149 ± 0.013	0.172–0.226	0.197 ± 0.015	0.149–0.226	0.182 ± 0.020
HL VIII	0.018–0.025	0.022 ± 0.002	0.018–0.025	0.022–0.003	0.010–0.021	0.019 ± 0.002	0.023–0.041	0.029 ± 0.005	0.026–0.048	0.032 ± 0.006
CAUDA	0.132–0.208	0.169 ± 0.018	0.122–0.160	0.144 ± 0.013	0.101–0.172	0.135 ± 0.013	0.153–0.182	0.169 ± 0.011	0.122–0.192	0.161 ± 0.016

Fig. 8. Bivariate plot of the lengths in mm of URS versus HTIB for alatae of *Schizaphis* spp. ($n=238$). Symbols: *S. graminum* (*), *S. holci* from *Holcus* (▼), trapped *S. holci* (●), trapped intermediate *Schizaphis* individuals (■), *S. agrostis* from *Agrostis* and *Poa* (♦), trapped *S. agrostis* (+).

Fig. 9. Bivariate plot of the lengths in mm of URS versus second segment of hind tarsus (HTII) for males of *Schizaphis* spp. ($n=57$). Symbols: *S. holci* reared in laboratory condition (♦), trapped *Schizaphis* individuals (▲), cotype of *S. agrostis* from *A. alba* (■).

separated from laboratory reared *S. holci* males (apterous or brachypterous with incompletely developed wings). The alate male cotype (syntype) of *S. agrostis* grouped with suction-trapped *Schizaphis* individuals. Based on this and because *S. agrostis* is reported to have alate males and *S. holci* apterous ones, all suction-trapped males are likely to be *S. agrostis*.

DNA sequence analysis

In total, 538 bases were obtained from the COI region for each of the 61 individuals tested from the UK. The number of bases was less than 640 because both the 5′ and 3′ ends were

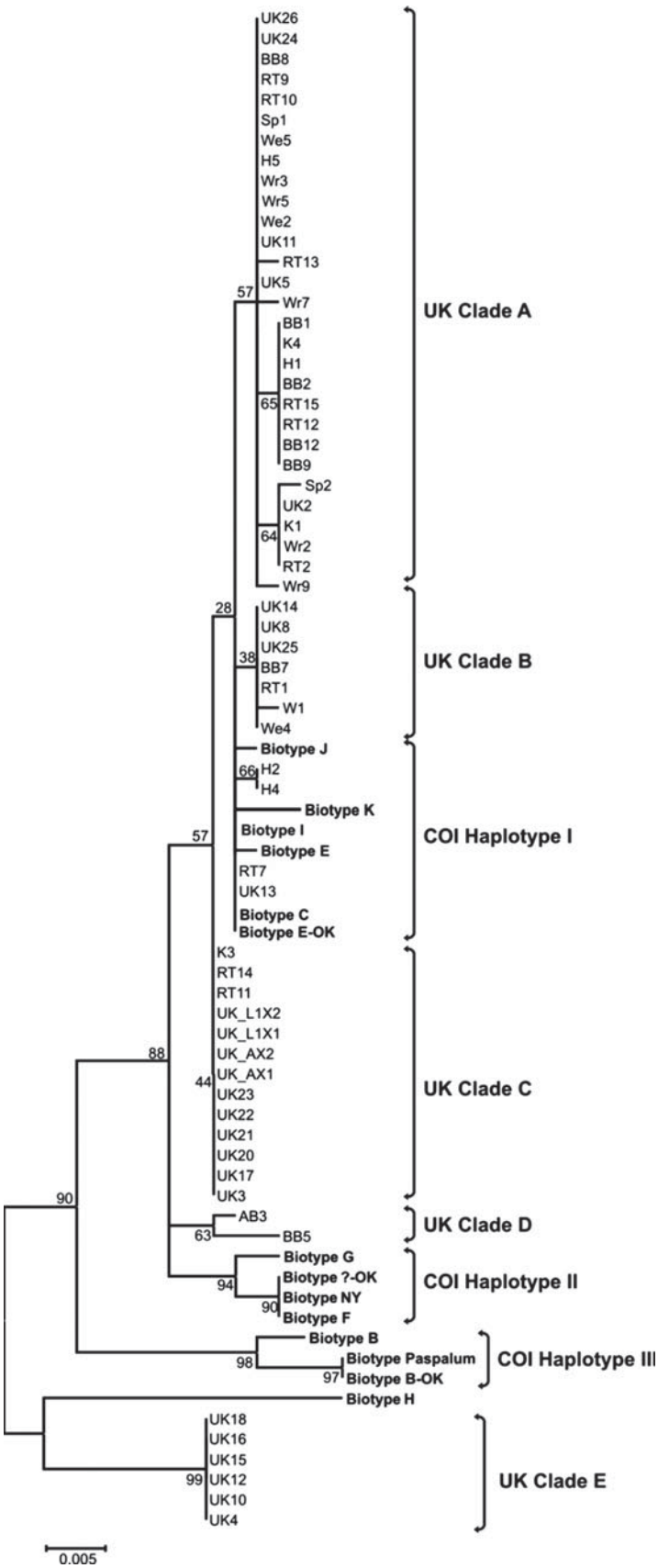

Fig. 10. Maximum likelihood tree of mtDNA COI sequences from specimens representative of *S. graminum* US biotypes (in bold) and specimens collected from the UK during 2009 and 2010.

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Table 10. Results of one-way ANOVA for each morphological character of alatae of *Schizaphis* spp. Variable names are defined in table 2.

Z	Source of variation	Sum of squares	df	Mean square	*F*	Significance	Wilks' Lambda	Significance
PT	Between groups	301171.666	5	60234.333	42.782	0.0000	0.520	0.0000
	Within groups	326638.624	232	1407.925				
	Total	627810.290	237					
ANTVIB	Between groups	21943.805	5	4388.761	43.120	0.0000	0.518	0.0000
	Within groups	23612.854	232	101.780				
	Total	45556.659	237					
ANTIII	Between groups	213175.299	5	42635.060	60.409	0.0000	0.434	0.0000
	Within groups	163739.861	232	705.775				
	Total	376915.160	237					
BDANTIII	Between groups	1213.580	5	242.716	64.255	0.0000	0.419	0.0000
	Within groups	876.357	232	3.777				
	Total	2089.937	237					
ROSTRUM	Between groups	301808.126	5	60361.625	70.406	0.0000	0.403	0.0000
	Within groups	204046.432	232	857.338				
	Total	505854.558	237					
URS	Between groups	11755.866	5	2351.173	144.157	0.0000	0.243	0.0000
	Within groups	3783.865	232	16.310				
	Total	15539.731	237					
HFEM	Between groups	591276.162	5	118255.232	136.357	0.0000	0.254	0.0000
	Within groups	201201.535	232	867.248				
	Total	792477.697	237					
HTIB	Between groups	2240276.230	5	448055.246	185.067	0.0000	0.200	0.0000
	Within groups	561681.703	232	2421.042				
	Total	2801957.933	237					
HTII	Between groups	35370.791	5	7074.158	228.574	0.0000	0.169	0.0000
	Within groups	7180.201	232	30.949				
	Total	42550.992	237					
SIPH	Between groups	133746.242	5	26749.248	86.505	0.0000	0.349	0.0000
	Within groups	71739.624	232	309.223				
	Total	205485.866	237					
HL VIII	Between groups	5777.195	5	1155.439	107.819	0.0000	0.301	0.0000
	Within groups	2486.217	232	10.716				
	Total	8263.412	237					
CAUDA	Between groups	51898.821	5	10379.764	38.865	0.0000	0.544	0.0000
	Within groups	61960.646	232	267.072				
	Total	113859.467	237					

trimmed to eliminate ambiguities. The UK and US specimens had COI sequence identities ranging from 95.4% to 100.0%. Two UK specimens (UK13 and RT7) had identical COI sequences with US *S. graminum* Biotypes C, E-OK and I. US Biotypes C, E and E-OK had 99.8% sequence identities (i.e., only differed by one base) with 28 UK specimens. Within the UK, specimens with identical sequences were found both within and between years (2009 and 2010), and both in suction traps and on *H. lanatus* (table 11).

The COI sequences of UK and US *Schizaphis* individuals were also compared by ML analysis and plotting of a consensus tree (fig. 10). The four US mtDNA COI haplotypes (I, II, III and H) are shown in fig. 10. The US biotypes with haplotypes II, III and H stood alone and their clades did not include any UK specimens.

A sister clade to H (UK Clade E) was well supported (99% bootstrap support) by six specimens collected at Rothamsted Research. These specimens were similar to H in that they were the most divergent of the UK samples. All UK specimens in UK Clade E had identical sequences and 95.9–96.5% and 96.3–96.8% sequence identities to US biotypes and the rest of the UK specimens, respectively. Five apterous specimens of US Biotype H preserved on slides in the NHML collection were re-examined and found to have URS/HTII ratio in the range of 0.77–0.89, which is characteristic of apterae of *S. agrostis*.

US Haplotype III grouped in its own clade outside of all UK specimens (fig. 10). Haplotype III biotypes had sequence identities of 96.1–98.5% with UK specimens. Haplotype II *S. graminum* from the US also formed a unique clade with no UK members and with sequence identities of 96.5–98.7%. Askham Bryan 3 and Broom's Barn 5 formed their own clade (UK Clade D) between the Haplotype II clade and the large clade containing Haplotype I and the majority of UK specimens (UK clades A, B and C).

There was 57% bootstrap support for the remainder of the tree, i.e., containing Haplotype I and UK specimens designated as clades A, B and C (fig. 10). Within this large clade, there was 44% bootstrap support for placement of 13 specimens with identical COI sequences as a sister clade to the rest of the group, and this was designated as UK Clade C. The topology of rest of the dendrogram was even less certain and contained the specimens that were most closely related to one another with COI sequence identities of 99.4–100%. UK Clade A was the best-supported clade in this part of the tree with 57% bootstrap support. Two subclades containing only UK specimens were located within UK Clade A. The US biotypes with Haplotype I grouped loosely together and within this group. Hereford 2 and 4 formed their own small subclade with 66% bootstrap support. UK clades A, B and C all included specimens from *Holcus*.

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Table 11. UK *Schizaphis* spp.: samples with identical (100% identity) mtDNA COI sequences. The taxonomic clade determined by ML analysis (fig. 10) is shown in relation to each identical sequence group. Samples with * were also identical to the US biotypes C, E-OK and I.

Sequence group	Sample ID	Location	Collection date	Where caught
I (UK Clade C)	K3	Kirton	22 June 2009	Suction trap
	UK21	Luton	25 June 2010	*H. lanatus*
	UK23	Luton	25 June 2010	*H. lanatus*
	UK_L1X1	Luton	25 June 2010	*H. lanatus*
	UK_L1X2	Luton	25 June 2010	*H. lanatus*
	RT11	Rothamsted	23 May 2009	Suction trap
	RT14	Rothamsted	01 June 2009	Suction trap
	UK3	Rothamsted	21 May 2010	Suction trap
	UK17	Rothamsted	20 June 2010	Suction trap
	UK20	Rothamsted	03 June 2010	*H. lanatus*
	UK22	Rothamsted	03 June 2010	*H. lanatus*
	UK_AX1	Rothamsted	02 June 2010	*H. lanatus*
	UK_AX2	Rothamsted	02 June 2010	*H. lanatus*
II (UK Clade E)	UK4	Rothamsted	22 May 2010	Suction trap
	UK10	Rothamsted	04 June 2010	Suction trap
	UK12	Rothamsted	05 June 2010	Suction trap
	UK15	Rothamsted	11 June 2010	Suction trap
	UK16	Rothamsted	17 June 2010	Suction trap
	UK18	Rothamsted	26 June 2010	Suction trap
III (UK Clade A)	BB8	Broom's Barn	31 May 2009	Suction trap
	H5	Hereford	31 May 2009	Suction trap
	RT9	Rothamsted	27 June 2009	Suction trap
	RT10	Rothamsted	11 July 2009	Suction trap
	SP1	Silwood Park	10 May 2009	Suction trap
	UK5	Rothamsted	23 May 2010	Suction trap
	UK11	Rothamsted	04 June 2010	Suction trap
	UK24	Rothamsted	15 June 2010	*H. lanatus*
	UK26	Rothamsted	10 June 2010	*H. lanatus*
	We2	Wellesbourne	25 May 2009	Suction trap
	We5	Wellesbourne	09 July 2009	Suction trap
	Wr3	Writtle	24 May 2009	Suction trap
	Wr5	Writtle	31 May 2009	Suction trap
IV (UK Clade B)	BB7	Broom's Barn	28 May 2009	Suction trap
	RT5	Rothamsted	04 June 2009	Suction trap
	UK8	Rothamsted	31 May 2010	Suction trap
	UK9	Rothamsted	02 June 2010	Suction trap
	UK14	Rothamsted	09 June 2010	Suction trap
	UK25	Rothamsted	02 June 2010	*H. lanatus*
	We 4	Wellesbourne	29 June 2009	Suction trap
V (UK Clade A)	BB1	Broom's Barn	01 May 2009	Suction trap
	BB 2	Broom's Barn	02 May 2009	Suction trap
	BB9	Broom's Barn	13 June 2009	Suction trap
	BB12	Broom's Barn	13 July 2009	Suction trap
	H1	Hereford	25 May 2009	Suction trap
	RT12	Rothamsted	24 May 2009	Suction trap
	RT15	Rothamsted	13 June 2009	Suction trap
VI (UK Clade A)	K1	Kirton	29 April 2009	Suction trap
	RT2	Rothamsted	15 May 2009	Suction trap
	Wr2	Writtle	14 May 2009	Suction trap
VII*	RT7*	Rothamsted	14 June 2009	Suction trap
	UK13*	Rothamsted	06 June 2010	Suction trap

In total, 518 bp of the nDNA CytC were obtained from samples UK13, RT7, H2 and H4. UK13 and RT7 were chosen for nDNA CytC intron sequencing because they had identical COI sequences to US *S. graminum*. H2 and H4 were chosen because they had 99.8% sequence identities to US biotypes C, E-OK and I, and because of their position in the dendrograms, i.e., H2 and H4, were a subclade within a larger clade that contained the US sorghum biotypes (C, E, I and K) (fig. 10). UK13 and RT7 had identical CytC sequences to US *S. graminum* Biotypes C, E, E-OK, I and K. H2 and H4 had identical CytC intron sequences to each other; however, they differed from the remaining US biotypes with sequence identities ranging from 97.5% to 99.8%. Based on CytC intron sequence identity, H2 and H4 were most closely related to US biotypes (99.8% identity) NY, F, G, ?-OK and H. Biotype J had the least sequence identity with H2 and H4 (97.5%); however, Biotype J had 99.4% identity with UK13 and RT7. The coding regions of the CytC gene were conserved. All CytC base substitutions occurred in the introns and none in the coding regions.

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Discussion

The results from the host choice and life history studies performed on the *Schizaphis* species found in the UK on *H. lanatus* suggest that the species involved was actually *S. holci* and hence unlikely to be a threat to crops. Nevertheless, even though *S. holci* did not show preference for barley as a host and did not establish a single colony on it, a few adults did start to feed and reproduce on it. This may be an indication that the aphid has the potential to switch hosts and colonize barley. It is conceivable that, as the experimental clones were collected from *H. lanatus* and apterae transferred to new experimental hosts, host-plant conditioning led to preference for *H. lanatus* as, in the wild, it would usually be alatae that effect host transfer.

The two UK *S. holci* lineages were shown to go through sexual reproduction under short-day conditions and produce fully apterous males and males with various states of brachyptery, but none with fully formed wings. This agrees with Hille Ris Lambers' (1947) and Stroyan's (1984) observations that males of *S. holci* are wingless, although very few specimens were examined by them and hence this may be misleading. It would be useful to search for males on *H. lanatus* in autumn to see whether wingless and brachypterous males of *S. holci* are produced under natural conditions. The same authors stated that males of *S. agrostis* are winged. Winged males were caught in the suction traps in autumn, and morphometric study indicated that these were *S. agrostis*, although *S. agrostis* could not be found in the field during a search of the Rothamsted grounds. Males of *S. graminum* are also winged (Webster & Phillips, 1912). No ant attendance was observed even though Stroyan (1984) stated that *S. holci* is usually attended by ants.

Based on morphometric investigation it is clear that the *Schizaphis* spp. trapped in the UK are mostly *S. agrostis* and *S. holci*, with *S. agrostis* being the most abundant in the years 2007 and 2011 according to those specimens so far examined. No individuals of *S. graminum* were found. However, only a small proportion of the trapped *Schizaphis* individuals have so far been examined using the techniques here described.

Schizphis graminum has never been found on crops in the UK, but it is clearly important to be alert for it. This study has provided the first morphometric analysis to facilitate discrimination of this species from its close relatives and is especially useful in the case of alate individuals for which no host plant information is yet available, such as those specimens collected by suction-trapping. Rather than undertaking a full morphometric analysis involving ten characteristics, it should be possible to establish the identity of most alate *Schizaphis* individuals in the UK by measuring the two characters URS and HTII or URS and HTIB and plotting their positions on the bivariate plots in figs 7 and 8 and thus ascertain presence or absence of *S. graminum*. The plot of URS versus HTII gives a clearer result than the plot of URS versus HTIB, but because suction-trapped aphids have often lost their hind tarsi, the plot of URS versus HTIB may be the only possibility. For measurement of URS, HTIB and HTII, it is necessary to prepare slides, as only HTIB can be measured accurately on whole, unmounted specimens, i.e., those not prepared on glass slides for microscopical examination.

Out of 62 specimens collected in the UK, only two collected at Rothamsted (RT7 and UK13) had identical mtDNA COI coding sequences and nDNA CytC intron sequences to specimens found in the US studies. As these two specimens had mtDNA and nDNA intron sequences identical to US biotype C, it is probable that they were *S. graminum*. Therefore, the specimen collected in the trap at Rothamsted on 14 June 2009 would represent the first record of *S. graminum* in the UK, and a second collection occurred at the same location on 6 June 2010.

Other individuals differed to varying extents from US biotypes of *S. graminum* (fig. 10). Whether these were different species cannot be determined based on COI sequences alone, but when taken together with the results of morphometric analysis certain conclusions are possible. 'UK Clade E' grouped apart from all other UK specimens as a sister group to the rare US Biotype H, and when apterae of this biotype on previously prepared slides were re-examined they were found to have morphological characteristics of *S. agrostis*, although this species has not hitherto been recognized as occurring in the USA. Probably then the six specimens comprising 'UK Clade E' are *S. agrostis*. UK Clades A, B and C all included specimens collected from *Holcus*, so are almost certainly *S. holci*. If so, the nesting of US Haplotype I within this grouping argues for a close affinity between this haplotype, which is characteristic of the sorghum-adapted form of *S. graminum*, and *S. holci*. However, this was not supported by the results of the morphometric analysis. The relationships in this part of the cladogram are in any case rather weakly supported, and several authors (e.g., Zhang & Hewitt 1996; Hurst & Jiggins 2005) have drawn attention to the problems of relying on COI sequences for studying inter-species relationships.

As *S. graminum* is known from southern Europe, including Spain, Italy and Greece, it is possible that individuals might at times reach the UK. Establishment is expected to be increasingly likely as the UK climate warms (Harrington *et al.*, 2001). On the other hand, species that were rare and therefore possibly overlooked are now becoming more abundant under the changes in temperature and climate (Hullé *et al.*, 2010), which may be the case for *S. holci* and *S. agrostis*. The two putative *S. graminum* specimens from Rothamsted had identical sequences to the sorghum Biotype C, which was found to be more tolerant of temperature extremes than Biotype B (Harvey & Hackerott, 1969; Harvey, 1971; Wood & Starks 1972). So far, it remains unknown whether the cereal varieties grown in the UK are suitable hosts for *S. graminum* from central or southern Europe.

In conclusion, to date, there have been no reports of *S. graminum* attacking cereal crops in the UK. As shown in the present study, based on morphometric analyses, field searches and experimentation, the majority of the *Schizaphis* individuals collected in suction-trap samples are likely to be *S. agrostis and S. holci*. We have shown that *S. holci* is unlikely to colonize barley, but cannot rule this possibility out. Nonetheless, the finding of two individuals in the UK which match precisely the COI and CytC of *S. graminum* raises concerns over a possible threat to crops. Alternatively, the COI and CytC sequences currently used to identify *S. graminum* are not completely reliable in distinguishing species. Even if the *Schizaphis* found in the UK are not *S. graminum sensu stricto*, the possibility of this species spreading from other parts of Europe remains a concern. *S. agrostis* and *S. holci* have, until now, been considered rare in the UK. Their remarkable population build up in recent years remains unexplained, although perhaps their previous rarity, despite their very abundant host plants, is more surprising.

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