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Morphological discrimination of *Aphis gossypii* (Hemiptera: Aphididae) populations feeding on Compositae

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Abstract

Aphis gossypii Glover is a polyphagous aphid pest with a worldwide distribution. However, there is evidence that on a global scale the name *A. gossypii* is being applied to a number of forms with different life cycles and/or host-plant associations. Morphometric variation of *A. gossypii* samples from crops and non-cultivated plants in many parts of the world was examined, to determine whether this variation is correlated with the hosts from which the aphids originated. Samples of *A. gossypii* were collected from Cucurbitaceae and Malvaceae in Europe, and from Compositae in various parts of the world. Morphometric data for 13 parameters measured from 97 clonal lineages (728 specimens) and 27 field-collected samples (313 specimens) were analysed by a series of canonical variates analyses, using the field sample/clonal lineage as grouping factor. Clonal lineages were reared on a common host in controlled conditions to standardize the effect of host and environment on morphology. The analyses provided a clear morphometric separation of the aphids originating from Compositae and those collected on Cucurbitaceae and Malvaceae, regardless of the geographical origin of the aphids and the host plant on which they were reared. This indicates that within *A. gossypii* there are two widely distributed host races or subspecies with different plant family associations. The taxonomic implications are discussed.

Keywords: *Aphis gossypii*, Compositae, Cucurbitaceae, Malvaceae, morphometric variation, host races, subspecies

Introduction

Intraspecific variation with respect to resource utilization is a common and well-known phenomenon in phytophagous insects (Mopper & Strauss, 1997). Many examples have been reported in insects of various orders: Diptera (e.g. Tephritidae: Feder *et al*., 1997; Itami *et al*., 1997), Lepidoptera (e.g. Yponomeutidae: Menken, 1996), Coleoptera (e.g. Chrysomelidae: Futuyma, 1990) and Hemiptera (Aleyrodidae: Legg, 1996; Delphacidae: Claridge *et al*., 1997). Among phytophagous insects, aphids are good candidates for host specialization. They show a high degree of host specificity, with 99% of all species colonizing one or a few closely-related plant species (Eastop, 1973) and it has been suggested that most aphid pests show intraspecific partitioning of resources (Blackman, 1990). Many polyphagous or oligophagous aphid species of the family Aphididae, e.g. *Acyrthosiphon pisum* (Harris) (Via, 1991a,b; Via & Hawthorne, 2002), *Aphis fabae* (Scopoli) (Mackenzie, 1996), *Cryptomyzus galeopsidis* (Kaltenbach) (Guldemond, 1990), *Myzus persicae*

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(Sulzer) (Blackman, 1987; Margaritopoulos *et al*., 2000; Nikolakakis *et al*., 2003), *Rhopalosiphum maidis* (Fitch) (Brown & Blackman, 1988), and *Sitobion avenae* (Fabricius) (De Barro *et al*., 1994; Lushai *et al*., 2002), comprise two or more subspecies, host races or biotypes with particular host-plant associations, often occurring sympatrically. Some of these intraspecific forms have been shown to exhibit genetically-determined differences in host preference and performance.

The cotton or melon aphid *Aphis gossypii* Glover is an interesting case because it is cosmopolitan and extremely polyphagous. It is a serious pest on field and glasshouse crops, especially cotton *Gossypium hirsutum* L. (Malvaceae), Cucurbitaceae as well as ornamental plants, e.g. *Chrysanthemum* sp. (Compositae) and *Hibiscus* sp. (Malvaceae). *Aphis gossypii* also causes indirect damage by transmitting more than 50 plant viruses and has developed widespread resistance to insecticides (Blackman & Eastop, 2000). The taxonomic status of *A. gossypii*, however, is rather complicated. In Europe, taxonomic works of Stroyan (1984) and Heie (1986) treated *A. gossypii* as a subspecies of the *Aphis frangulae* Kaltenbach complex, a group of closely-related indigenous European species, almost morphologically indistinguishable, which have different hosts in their parthenogenetic phase but all migrate for their sexual phase to the European alder buckthorn *Rhamnus frangula* L. (Rhamnaceae). *Aphis gossypii* is considered as the only member of the group that does not have a sexual phase on *Rhamnus*, reproducing continuously by apomictic parthenogenesis on a wide range of herbaceous host plants. However, it has been found that *A. gossypii* from both chrysanthemum *Dendranthema grandiflora* Tevzel (Compositae) (Guldemond *et al*., 1994) and cucumber *Cucumis sativus* L. (Cucurbitaceae) (Fuller *et al*., 1999) in western European glasshouses can produce sexual morphs under short-day conditions.

The biology of aphids regarded as *A. gossypii* in certain other parts of the world is quite different. In North America, Kring (1959) demonstrated that *A. gossypii* has an annual sexual phase utilizing southern catalpa *Catalpa bignonioides* Walter (Bignoniaceae) and rose of Sharon *Hibiscus syriacus* L. (Malvaceae) as primary hosts. In China and Japan, populations of *A. gossypii* can also reproduce sexually, migrating from herbaceous plants to various unrelated primary hosts including *Rhamnus* spp., flatspine prickly ash *Zanthoxylum simulans* Hance (Rutaceae), pomegranate *Punica granatum* L. (Punicaceae), oriental bittersweet *Celastrus orbiculatus* Thunb. (Celastraceae), and Indian madder *Rubia cordifolia* L. (Rubiaceae) (Inaizumi, 1980; Zhang & Zhong, 1990). Zhang & Zhong (1990) also reported non-host-alternating populations of *A. gossypii* on *H. syriacus*.

Other evidence that *A. gossypii* cannot be considered as a genetically homogeneous species has been revealed from host transfer experiments. Furk & Hines (1993) reported that aphids from chrysanthemum in UK glasshouses did not colonize cucumber and vice versa. Similarly, Guldemond *et al*. (1994), performing reciprocal host transfer experiments, found that parthenogenetic lineages from chrysanthemum in glasshouses in the Netherlands have little or no reproduction on cucumber and vice versa, and suggested that aphids from chrysanthemum and cucumber are genetically distinct host races. The association of *A. gossypii* genotypes with certain hosts has also been reported in Asian populations. Zhang & Zhong (1990) demonstrated that two anholocyclic forms, a green and a yellow one, performed better on cucumber than on other cucurbitaceous plants, but the green form, in particular, would not colonize cotton. By contrast, heteroecious holocyclic aphids derived from *Z. simulans* performed much better on cotton than on any Cucurbitaceae. Similarly, Moursi *et al*. (1985) found that *A. gossypii* from cotton reproduced more slowly on eggplant *Solanum melongena* L. (Solanaceae), sweet melon *Cucumis melo* L. (Cucurbitaceae), okra *Abelmoschus esculentus* (L.) (Malvaceae) and sesame *Sesamum indicum* L. (Pedaliaceae) than on cotton. Also, different levels of insecticide resistance, observed between populations (Takada & Murakami, 1988), are in some cases related to the host plant from which the aphids were collected (e.g. Furk *et al*., 1980; Saito, 1991).

Recently, further evidence of genetic heterogeneity within *A. gossypii* has emerged from studies involving DNA analysis. Random amplified polymorphic DNA (RAPD) markers (Vanlerberghe-Masutti & Chavigny, 1998) revealed genetic structuring within *A. gossypii* according to host plant. The 18 populations examined from southern France, La Réunion, Portugal and Laos were separated into two groups, i.e. populations from cucurbits and those from non-cucurbits. These authors, and Martinez-Torres *et al*. (1997) using the same method, showed that the populations of *A. gossypii* examined from Cucurbitaceae and Rutaceae, respectively, were multiclonal.

Given these findings, it is important to try to establish whether *A. gossypii* lineages exhibiting different host associations are diverging, becoming distinct taxonomic entities. If this is so then they might show consistent morphological differences.

In the present paper a multivariate discriminant technique (canonical variates analysis, CVA) was applied to numerous laboratory-reared clonal lineages and field samples of *A. gossypii* collected from a broad range of localities around the world, years of collection and host-plants (Compositae, Cucurbitaceae and Malvaceae), to test whether there were host-related genetic differences. In discussing the results the taxonomic status of populations of *A. gossypii* inhabiting different plants is considered.

Materials and methods

The study was based on aphids collected from Greece and Serbia during 2002–2004 and also on material (permanent slides) from the collection of The Natural History Museum, London (NHM) consisting of samples from different parts of the world collected during the last four decades (table 1). The aphids were collected from various crops and uncultivated plant species of the families Compositae (*Chrysanthemum* sp., smooth sow-thistle *Sonchus oleraceus* L., dahlia *Dahlia variabilis* (Willd.), *Vernonia* sp., groundsel *Senecio vulgaris* L., *Crassocephalum crepidioides* (Bentham), African marigold *Tagetes erecta* L., Siam weed *Eupatorium odoratum* L., narrow-leaved zinnia *Zinnia angustifolia* Kunth, safflower *Carthamus tinctorius* L., and floss-flower *Ageratum conyzoides* L.), Cucurbitaceae (squirting cucumber *Ecballium elaterium* (L.), watermelon *Citrullus lanatus* (Thunb.), sweet melon, zucchini *Cucurbita pepo* L. and cucumber) and Malvaceae (cotton, okra, *H. syriacus* and common mallow *Malva sylvestris* L.).

The studied material consisted of 1041 adult apterous parthenogenetic females (virginoparae), 728 from 97 clonal lineages (94 from Greece and three from UK) and 313 from 27 field samples (23 from NHM and four from Serbia). The

Table 1. Field samples and laboratory-reared parthenogenetic lineages of *Aphis gossypii* examined in the present study.

Plant	Locality	Date	No. lineages or field samples	No. specimens
Compositae				
Chrysanthemum sp.	Central Greece	1.ix.03	4	48
Chrysanthemum sp.	Central Greece	27.ix.03	3	30
Chrysanthemum sp.	Central Greece	15.x.03	2	21
Chrysanthemum sp.	England, UK	16.v.76	1[a]	19
Chrysanthemum sp.	England, UK	25.v.76	1*	9
Chrysanthemum sp.	England, UK	27.xi.78	1*	11
Chrysanthemum sp.	England, UK	22.xi.87	1*	8
Chrysanthemum sp.	England, UK	6.ix.79	1*	12
Chrysanthemum sp.	England, UK	6.ix.79	1*	17
Chrysanthemum sp.	England, UK	25.vii.79	1*	31
Chrysanthemum sp.	England, UK	21.v.76	1*	5
Chrysanthemum sp.	Gambia	24.xi.93	1*	10
Sonchus oleraceus	Central Greece	9.ix.03	4	39
Sonchus oleraceus	Central Greece	10.v.04	4	23
Dahlia variabilis	Central Greece	15.x.03	1	10
Vernonia sp.	Kenya	14.viii.70	1*	6
Senecio vulgaris	Lebanon	10.ii.72	1*	10
Crassocephalum crepidioides	New Guinea	5.x.79	1*	10
Crassocephalum crepidioides	New Guinea	29.ix.79	1*	10
Tagetes erecta	Pakistan	7.i.63	1*	10
Eupatorium odoratum	Thailand	1966	1*	10
Zinnia angustifolia	Suriname	8.xii.64	1*	10
Carthamus tinctorius	Brazil	11.x.71	1*	11
Carthamus tinctorius	Brazil	16.vi.71	1*	3
Ageratum conyzoides	Philippines	10.ii.55	1*	10
Cucurbitaceae				
Ecballium elaterium	Central Greece	5.ix.02	1	9
Citrullus lanatus	North Greece	8.viii.02	5	54
Cucumis melo	North Greece	8.viii.02	6	45
Cucumis melo	Serbia	25.viii.04	3*	30
Cucurbita pepo	Central Greece	5.viii.02	9	59
Cucurbita pepo	Central Greece	14.viii.02	4	38
Cucumis sativus	Central Greece	30.viii.02	1	6
Cucumis sativus	England, UK	13.ii.89	1[a]	14
Cucumis sativus	England, UK	16.ix.87	1*	17
Cucumis sativus	England, UK	26.x.78	1*	20
Cucumis sativus	England, UK	25.vii.79	1*	13
Cucumis sativus	England, UK	6.ix.79	1*	11
Cucumis sativus	England, UK	ii.72	1[b]	11
Cucumis sativus	England, UK	13.ii.89	1	9
Malvaceae				
Gossypium hirsutum	North Greece	8.viii.02	17	94
Gossypium hirsutum	Central Greece	2.viii.02	15	100
Abelmoschus esculentus	North Greece	8.viii.02	12	77
Hibiscus syriacus	Central Greece	29.viii.02	4	24
Hibiscus syriacus	Serbia	25.v.04	1*	10
Malva sylvestris	Central Greece	26.viii.02	2	17

Field samples are indicated with an asterisk (*). All Greek lineages were laboratory reared on pepper and three from the UK on [a]cotton and [b]cucumber.

Greek clonal lineages were reared under constant conditions (L16 : D8 at 17°C) on excised leaves of pepper *Capsicum annum* L. (Solanaceae) in Blackman boxes (Blackman, 1971) for 2–3 generations before preserving, clearing and mounting specimens on microslides for measurement. Two lineages (18 specimens) were also measured after they had been reared on pepper for approximately five months (about 15 parthenogenetic generations). The NHM clonal material comprised two lineages originating from cucumber in the UK (one had been reared on cotton and the other on cucumber), and one lineage originating from chrysanthemum and reared on cotton, all in glasshouse conditions (uncontrolled environment). Clonal lineages were set up from single adult apterous viviparous females collected from different randomly-selected plants, while each field sample consisted of adult apterae collected from the same leaf or plant on the same date. Chrysanthemums in the UK and Greece as well as dahlia in Greece and cucumber in the UK were cultivated in glasshouses (table 1). Each sample from cucumber and chrysanthemum from the UK was collected from a different glasshouse, while the chrysanthemum samples from Greece derived from three glasshouses. The other aphid samples

from crops (cotton, cucurbits, okra) in Greece and Serbia were collected from one field for each sampling date. Aphids from Greece and Serbia were preserved in tubes filled with one part lactic acid (75% w/w) and two parts ethyl alcohol (95%), until slide preparation. They were mounted on slides according to the method of Blackman & Eastop (2000).

Usually, 7–10 specimens (minimum 3, maximum 31) of each clonal lineage or field sample were measured. The following 13 characters were measured on each specimen: length of fifth antennal segment (ant v), length of base of sixth antennal segment (base vi), length of terminal process of sixth antennal segment (pt), length of ultimate rostral segment (urs), length of hind femur (hf), length of middle femur (mf), length of front femur (ff), length of hind tibia (ht), length of middle tibia (mt), length of front tibia (ft), length of second segment of hind tarsus (ht ii), length of siphunculus (ls) and length of cauda (lc) (for details of measurements see Ilharco & van Harten, 1987). All measurements were carried out with a phase contrast microscope (Leica DRMB, Leica Mikroskopie und System GmBH, Germany) using a calibrated micrometer eyepiece.

To examine whether host-related differences existed among lineages and field samples, the data were submitted to a canonical variates analysis (CVA) without any prior transformation (Krzanowski, 1990). Each lineage or field sample was treated as one group in the analysis. Using this procedure the analysis becomes completely objective as no information is included about potential interrelationships of the groups. Thus, a common criticism (Thorpe, 1983) of the use of CVA in systematic studies is overcome. Correlations between vectors provided by CVA and a general size index (= sum of the lengths of all characters measured) were examined as a possible guide to the relative contributions of genetic and environmental components in the morphological separation among samples. CVA was performed using the SPSS ver.10 (SPSS Inc., 1999) statistical package and some basic statistics using STATISTICA 6.0 (StatSoft Inc., 2001).

CVA is believed to be sensitive to heteroscedasticity (different within-group variances), and samples with heterogeneous variances could be misplaced or could affect the whole analysis (Thorpe, 1983). It is likely that field samples are more heterogeneous than clonal lineages reared in controlled conditions where environmental effects are eliminated or at least minimized. The homogeneity of variance was therefore examined in field samples and clonal lineages reared in the laboratory but not in controlled conditions using Brown & Forsythe's test (Brown & Forsythe, 1974).

Fisher's linear discriminant functions (LDFs) (Fisher, 1936) were used to examine the correct classification of individual aphids into well defined groups obtained by CVA. To avoid possible circularity of applying a discriminant function to the data from which it was obtained, the specimens were randomly partitioned into two subsets: the training set from which LDFs were calculated and the test set on which the functions were applied in order to evaluate their reliability (the method is discussed further in Blackman & Paterson, 1986). Furthermore, LDFs were calculated from the whole dataset in order to take into account all the variation of the data. In both cases, a stepwise method was used by excluding variables which contributed less to the separation of the two groups.

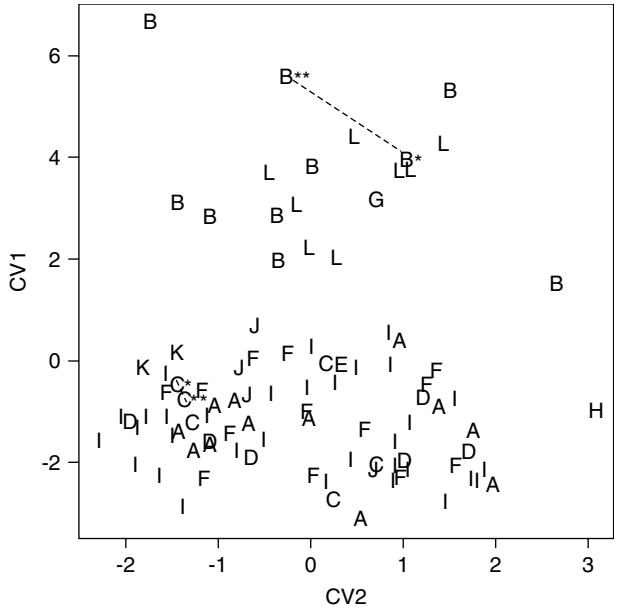

Fig. 1. Plot of the mean scores on the first two canonical variates for 94 lineages of *Aphis gossypii* collected from Malvaceae, Cucurbitaceae and Compositae plants in Greece. The lineages were reared on pepper at 17°C and L16 : D8 for 2–3 generations before measurements. A, *Abelmoschus esculentus*; B, *Chrysanthemum* sp.; C, *Citrullus lanatus*; D, *Cucumis melo*; E, *Cucumis sativus*; F, *Cucurbita pepo*; G, *Dahlia variabilis*; H, *Ecballium elaterium*; I, *Gossypium hirsutum*; J, *Hibiscus syriacus*; K, *Malva sylvestris*; L, *Sonchus oleraceus*. Dotted lines join sub-samples of the same lineage measured after a short (B*, C*) and a long term (B**, C**) parthenogenetic rearing in the laboratory.

Results

Clonal lineages from Greece reared on pepper in constant conditions

In the first CVA only the aphids from Greece collected during 2002–2004 were analysed, comprising 94 parthenogenetic lineages reared on excised leaves of pepper in constant conditions. Thus, the physical environment was standardized across all clonal lineages and any variation detected in morphology could be attributed to genotypic differences, with possibly some effect also due to differences in nutritional quality of the excised leaves. Figure 1 is a plot of means of the first two canonical variates (CVs), which together accounted for the 66% of the total variance of the data. The lineages formed two distinct clusters, and all the clones originating from Compositae were separated from those on Cucurbitaceae and Malvaceae by their mean scores on CV1, which accounted for 52% of the total variance. One lineage from *Chrysanthemum* sp. (Compositae) and one from *Ecballium elaterium* (Cucurbitaceae) were separated from the main clusters by their CV2 scores. The sub-samples of two lineages from *Chrysanthemum* sp. and watermelon that were measured after rearing for five months on pepper were located in the same cluster with the originals, although some change in CV scores was observed. The characters with the highest standardized coefficients of CV1 were pt, ant v, ls and urs (table 2). Both vectors were significantly, although not highly, correlated with a general size index (table 2). Size

variation was probably chiefly attributable to genotypic differences as the rearing system minimized the effects of environment on phenotype, although there could be some effect of variations in host plant condition, which cannot be controlled completely even by the use of excised leaves.

UK samples

Aphids from the NHM collection, comprising 12 samples collected from chrysanthemum and cucumber in UK glasshouses and three parthenogenetic lineages reared on cotton and cucumber (but not at constant conditions), were analysed. In this CVA (fig. 2) the aphids originating from chrysanthemum were clearly separated from those from cucumber by their scores on CV1, which accounted for 49.1% of the total variance of the data. Lineages reared on cotton clustered according to the host plant on which they originated, showing that the separation according to host by CV1 was due to genetic differences rather than to the effect of host on phenotype. The morphometric characters with the highest CV1 coefficients were ff, urs, mt and pt (table 2). CV2 (accounting for 30.6% of the variance) separated the aphids from chrysanthemum into two groups according to their CV2 scores. CV2 values were rather highly correlated with the size index (table 2). The three samples from chrysanthemum with the lowest CV2 values were much smaller aphids. For instance, the mean lengths of hind femur in these three samples were 48–62%, 54–70% and 59–77% (for each sample respectively) of that of all other samples. Thus, it seems probable that CV2 in this analysis is associated with unknown environmental factors causing size differences between samples.

Field samples from various parts of the world

To see whether the separation of aphids from Compositae holds when further samples are examined, three field samples from melon and one from *Hibiscus syriacus* from Serbia (collected in 2004) and 11 field samples from a range of Compositae from three continents collected during the last four decades were incorporated into the previous dataset. In the plot with the first two vectors (fig. 3), which together account for 65.5% of the total variance, there appears again to be a separation between aphids from Compositae and those from other plants, in spite of the wide range of localities, sampling years and host plants. Both CV1 and CV2 contribute to separation of the two groups. The morphometric characters with the highest CV1 coefficients were ff, mt, pt and urs and those with the highest CV2 coefficients were ff, pt, urs and ft (table 2); although three of these characters are common to both CVs, the signs of the coefficients were different. CV1 separated Compositae and non-Compositae clusters except the samples from *Senecio vulgaris* and *Hibiscus syriacus* from Lebanon and Serbia respectively, which showed almost identical values for both vectors and were located away from the two clusters. CV1 in this analysis was highly size-correlated, probably reflecting an increased contribution from environmental factors (table 2; and note that the three samples of very small UK aphids have different CV1 values from other UK aphids, compared with similar values for this CV in fig. 2). Most of the samples from Compositae showed higher CV2 scores than those from Cucurbitaceae. Two samples from *Carthamus tinctorius* from Brazil and one from *Eupatorium odoratum* from Thailand were located near the Cucurbitaceae cluster.

Using Brown & Forsythe's test (1974), the variance was found to be homogenous among samples in urs, base vi, pt, ls, ht and hf, while heterogeneity was observed in ht ii, ant v, lc, mt, mf, ft, ff. Coefficients of variation were also calculated for all characters and samples in the analysis, i.e. 390 cases. In most cases (89.7%) the coefficient of variation was less than 15.0%, while only in 2.3% of the cases was it high, i.e. 20.0–35.0%. These results showed that there are few high variances and therefore the structure of the data is unlikely to have a significant effect on CVA.

Field samples plus clonal lineages reared at constant conditions

In the next analysis clonal lineages originating from cotton, okra, zucchini and chrysanthemum in Greece were included along with the samples of the previous data set, but three UK samples of very small specimens from chrysanthemum were excluded (3, 4 and 13 in fig. 3, see also fig. 2), because their size could reduce the resolving power of the analysis.

In this analysis, CV2 (accounting for 29.4% of the total variance) was not considered as it was highly correlated with the size index (table 2), and therefore probably had a strong environmental component. However, the plot of CV1 versus CV3 (accounting for 33.4 and 12.6% of the total variance, respectively) revealed two major clusters (fig. 4). The first cluster consisted only of aphid samples and lineages from cultivated and non-cultivated Compositae. The sample from *Senecio vulgaris* in Lebanon was located this time within the Compositae group (cf. fig. 3). Furthermore, the sample from *Eupatorium odoratum* in Thailand due to its low score on CV3 was not clustered with aphids from Cucurbitaceae and Malvaceae (cf. fig. 3). The second contained aphid samples and lineages from Cucurbitaceae and Malvaceae but also included the two samples from *Carthamus tinctorius* from Brazil that had CV1 and CV2 scores closest to the Cucurbitaceae cluster in the previous analysis (fig. 3).

The separation of these two clusters was due to the scores of CV1. The morphological characters with the highest coefficients for CV1 were pt, ff, urs and ls. All these characters had contributed significantly to the separation of the Compositae and non-Compositae groups in two or more previous analyses (table 2). The compactness of the second cluster (aphids from Malvaceae and Cucurbitaceae) with similar values of CV1 and CV3 for all samples suggests that this is a genetically homogeneous group in which the environmental effects on morphology have been effectively minimized; note that neither of these CVs is size-correlated (table 2).

Clustering within aphids from Compositae and non-Compositae

Two additional CVAs were carried out, examining separately the aphids from Compositae and those from Malvaceae and Cucurbitaceae. In this way, the resolving power of the method could be increased so as to detect any additional clustering within samples from Compositae and from Cucurbitaceae–Malvaceae. In the analysis including only the samples from Compositae, scores on CV1 (not shown) accounted for 41.0% of the total variance but

Table 2. Canonical coefficients (standardized) and percentage of total variance accounted by vectors which contributed to the separation of the aphid samples and lineages examined by the six canonical variates analyses (CVAs) performed.

Characters	First CVA (fig. 1)		Second CVA (fig. 2)		Third CVA (fig. 3)		Fourth CVA (fig. 4)			Fifth CVA (fig. 5)			Sixth CVA (see text)		
	CV1	CV2	CV1	CV2	CV1	CV2	CV1	CV2	CV3	CV1	CV2	CV3	CV1	CV2	CV3
urs	0.441	−0.158	−1.156	0.346	−0.621	0.656	0.783	−0.410	−0.296	0.208	−0.246	−0.099	−0.271	−0.442	−0.112
base vi	0.073	0.027	0.019	−0.106	−0.073	−0.036	0.041	−0.071	0.375	0.086	−0.429	0.054	0.018	0.050	0.427
ht ii	0.379	0.172	0.038	0.829	0.329	0.459	0.314	0.678	−0.111	0.449	0.405	0.251	0.518	−0.090	0.430
ant v	0.898	−0.250	0.283	−0.001	0.060	0.115	0.317	0.221	0.508	0.410	−0.413	−0.204	0.408	−0.191	0.687
pt	−1.021	0.895	0.861	−0.317	0.660	−0.929	−1.105	0.175	0.079	−0.691	0.644	0.966	−0.232	1.069	−0.603
lc	0.133	0.582	0.302	0.320	0.233	0.094	−0.063	0.053	0.547	0.385	−0.200	−0.121	−0.005	0.589	0.226
ls	−0.746	−0.013	0.814	−0.433	0.543	−0.296	−0.692	0.372	−1.854	−0.447	1.265	−1.402	0.587	−0.367	−0.348
ht	0.093	0.233	0.335	0.003	0.197	0.007	−0.061	0.016	0.241	0.093	−0.137	−0.195	0.097	0.165	−0.403
hf	0.288	−0.237	0.131	0.020	0.149	−0.016	−0.061	0.097	0.086	0.629	−0.628	0.728	0.075	0.092	−0.095
mt	−0.029	0.447	1.132	0.241	0.673	−0.315	−0.250	0.167	0.281	0.105	0.141	0.006	0.069	0.593	0.071
mf	−0.004	−0.651	−0.155	0.011	−0.03	0.186	0.133	−0.003	0.409	0.082	−0.261	0.228	−0.253	−0.617	−0.912
ft	0.255	0.058	0.335	−0.853	−0.279	−0.619	−0.476	0.007	0.203	0.242	−0.173	−0.229	−0.008	0.416	1.573
ff	−0.114	−0.039	−2.268	0.865	−0.808	1.181	1.157	−0.245	−0.165	−0.570	0.460	0.307	−0.011	−0.775	−0.898
% of total variance	51.8	14.6	49.1	30.6	36.0	29.5	33.4	29.4	12.6	41.0	15.6	13.3	49.2	16.1	9.0
Correlation with the size index	R = 0.74, $P < 0.01$	R = 0.63, $P < 0.01$	R = 0.22, $P < 0.08$	R = 0.73, $P < 0.01$	R = 0.84, $P < 0.01$	R = 0.43, $P < 0.02$	R = 0.19, $P < 0.30$	R = 0.92, $P < 0.01$	R = 0.09, $P < 0.60$	R = 0.90, $P < 0.01$	R = 0.30, $P < 0.07$	R = 0.22, $P < 0.19$	R = 0.97, $P < 0.01$	R = 0.19, $P < 0.08$	R = −0.09, $P < 0.42$

ant v, length of fifth antennal segment; base vi, length of base of sixth antennal segment; ff, length of front femur; ft, length of front tibia; hf, length of hind femur; ht, length of hind tibia; ht ii, length of second segment of hind tarsus; lc, length of cauda; ls, length of siphunculus; mf, length of middle femur; mt, length of middle tibia; pt, length of terminal process of sixth antennal segment; urs, length of ultimate rostral segment.

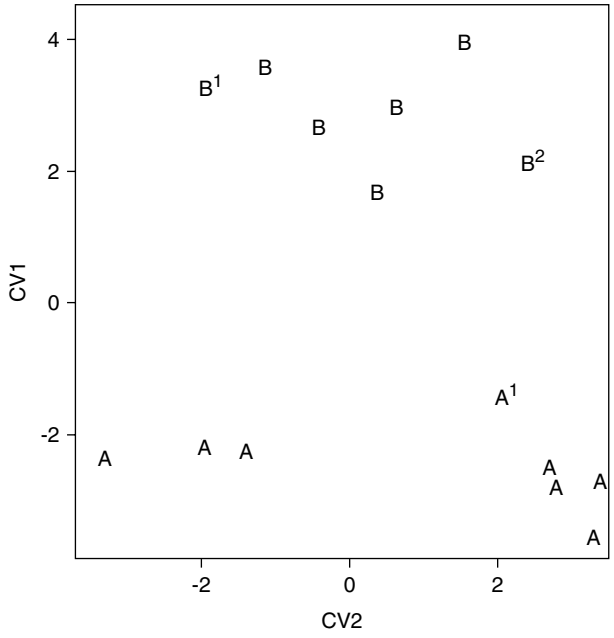

Fig. 2. Plot of the mean scores on the first two canonical variates for 12 field samples and three laboratory-reared ([1]on cotton and [2] on cucumber, but not at constant conditions) lineages of *Aphis gossypii* collected from cucumber (B) and chrysanthemum (A) in glasshouses in the UK.

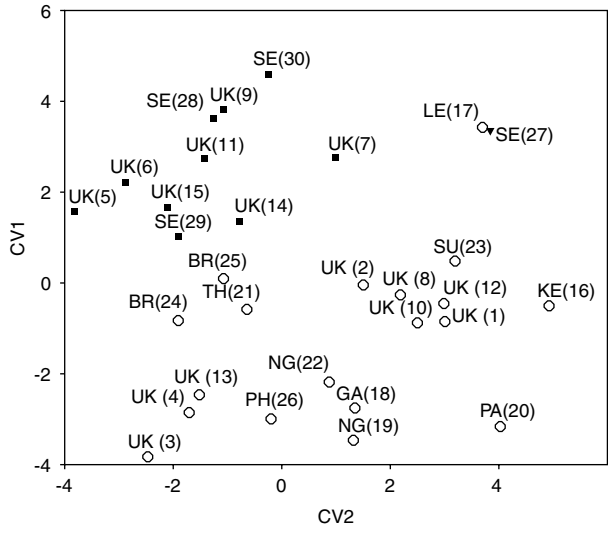

Fig. 3. Plot of the mean scores on the first two canonical variates for 27 field samples and three laboratory-reared (UK2 and UK5 on cotton and UK7 on cucumber, but not under controlled conditions) lineages of *Aphis gossypii* collected from Malvaceae (▼), Cucurbitaceae (■), and Compositae (○) plants from different regions of the world. 1–4, 8, 10, 12, 13, 18, *Chrysanthemum* sp.; 5–7, 9, 11, 14, 15, *Cucumis* sativus; 16, *Vernonia* sp.; 17, *Senecio vulgaris*; 19, 22, *Crassocephalum crepidioides*; 20, *Tagetes erecta*; 21, *Eupatorium odoratum*; 23, *Zinnia angustifolia*; 24–25, *Carthamus tinctorius*; 26, *Ageratum conyzoides*; 27, *Hibiscus syriacus*; and 28–30, *Cucurbita pepo*. BR, Brazil; GA, Gambia; KE, Kenya; LE, Lebanon; NG, New Guinea; PA, Pakistan; PH, Philippines; SU, Suriname; SE, Serbia; TA, Thailand; UK, United Kingdom.

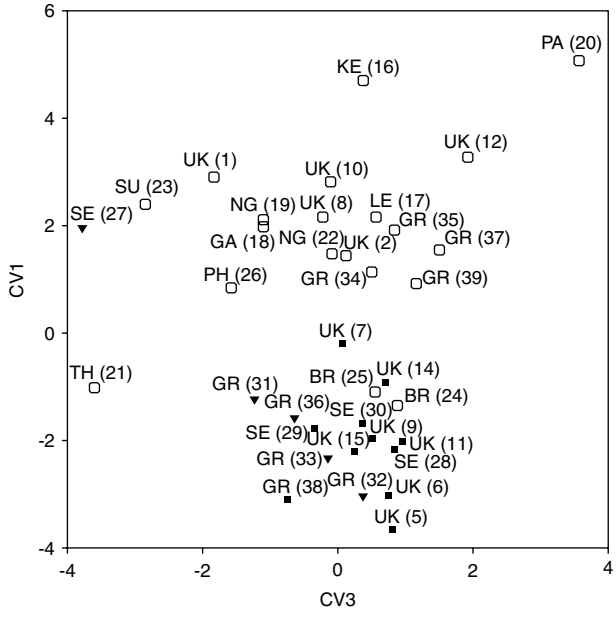

Fig. 4. Plots of the mean scores on the first and third canonical variate for 24 field samples and 12 laboratory-reared (2 and 5 on cotton, 7 on cucumber and 31–39 on pepper) lineages of *Aphis gossypii* collected from Malvaceae (▼), Cucurbitaceae (■), and Compositae (○) plants from different regions of the world. 1, 2, 8, 10, 12, 18, 34, 35, *Chrysanthemum* sp.; 5–7, 9, 11, 14, 15, *Cucumis sativus*; 16, *Vernonia* sp.; 17, *Senecio vulgaris*; 19, 22, *Crassocephalum crepidioides*; 20, *Tagetes erecta*; 21, *Eupatorium odoratum*; 23, *Zinnia angustifolia*; 24–25, *Carthamus tinctorius*; 26, *Ageratum conyzoides*; 27, *Hibiscus syriacus*; 28–30, *Cucumis melo*; 32, 36, *Abelmoschus esculentus*; 31, 33, *Gossypium hirsutum*; 37, *Sonchus oleraceus*; 38, *Cucurbita pepo*; and 39, *Dahlia variabilis*. BR, Brazil; GA, Gambia; GR, Greece; KE, Kenya; LE, Lebanon; NG, New Guinea; PA, Pakistan; PH, Philippines; SU, Suriname; SE, Serbia; TA, Thailand; UK, United Kingdom.

showed no evidence of clustering and appeared to be highly correlated with general size, indicating strong environmental influence (CV1 for fifth CVA in table 2: R = 0.90). However, a plot (fig. 5) of CV2 and CV3 (accounting for 15.6 and 13.3% of the total variance, respectively), which were not size-dependent (table 2), revealed a large cluster of mainly European samples with high scores on CV3, and most samples from other parts of the world separated from this by their scores on either CV2 or CV3. The aphids from Brazil in this analysis were located within the main European cluster, although they had grouped with non-Compositae samples in the previous analysis.

The final CVA comprised aphids from Cucurbitaceae and Malvaceae in Greece, Serbia and UK, including both reared clonal lineages and field-collected samples. Scores on CV1 (not shown) accounted for 49.2% of the total variance and were all similar for Greek lineages reared in a controlled environment but varied greatly in field-collected samples and those reared under uncontrolled (glasshouse) conditions. They were also highly size-correlated (sixth CVA in table 2), so it can be assumed that CV1 was associated with environmental differences between samples. A plot (not shown) of CV2 versus CV3, (accounting for 16.1 and 9.0% of the total variance respectively, neither of which were

size-dependent, see sixth CVA in table 2), did not reveal any clustering associated with either plant species/genus/family or sampling locality. Only one large cluster of samples was formed with a few outliers, including some (from *Ecballium elaterium* in Greece and from *Hibiscus syriacus* in Serbia) that were outliers in the first analysis (fig. 1).

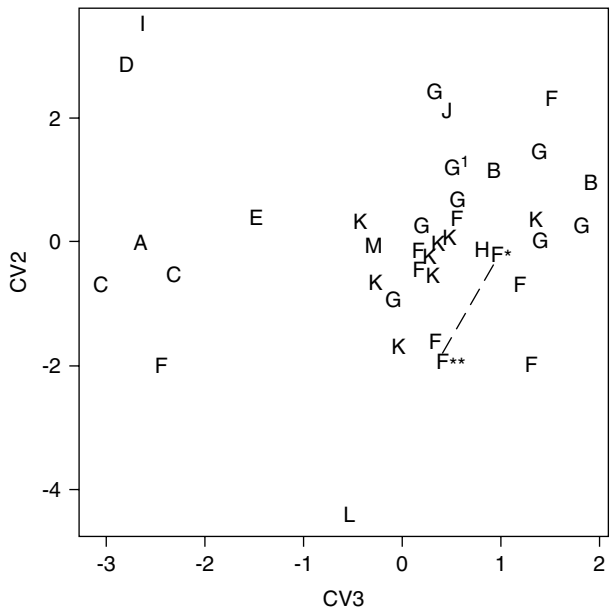

Fig. 5. Plot of the mean scores on the second and third canonical variate for 18 field samples (A–E, G, I, J, L, M) and 19 laboratory reared lineages of *Aphis gossypii* collected from Compositae plants from different parts of the world. A, *Ageratum conyzoides* (Philippines); B, *Carthamus tinctorius* (Brazil); C, *Crassocephalum crepidioides* (New Guinea); D, *Zinnia angustifolia* (Suriname); E, *Chrysanthemum* sp. (Gambia); F, *Chrysanthemum* sp. (Greece); G, *Chrysanthemum* sp. (United Kingdom); H, *Dahlia* (Greece); I, *Eupatorium odoratum* (Thailand); J, *Senecio vulgaris* (Lebanon); K, *Sonchus oleraceus* (Greece); L, *Tagetes erecta* (Pakistan); M, *Vernonia* sp. (Kenya). Greek lineages (F, H, K) were reared on pepper at 17°C and L16 : 8 and the UK lineage (G^1) on cotton but not under controlled conditions. Dotted line joins sub-samples of the same lineage measured after a short (F*) and a long (F**) term parthenogenetic rearing.

Fisher's linear discriminant functions and bivariate plots

Given that CVA reveals two major groups among the field samples and lineages of *A. gossypii* examined, a final step of analysis was to evaluate the separation of the Compositae and non-Compositae groups at the level of individual aphids by calculating Fisher's linear discriminant functions (LDFs). The LDFs calculated are listed in table 3. The percentage of correctly classified individuals in LDFs derived both from the training subset and the whole data set was 87.3%.

Combinations of pairs of characters were tested by plotting measurements for all individuals in bivariate plots. The best two-character discrimination between aphids from Compositae and Cucurbitaceae/Malvaceae was achieved in plots of urs vs. pt and urs vs. ls (fig. 6). Although there is a large overlap, particularly in the case of small specimens, these plots can be used to determine the co-ordinates of these characters for newly measured individuals, and thus provide a simple visual estimation of their identity. In the fig. 6 plots measurements are included of an aptera from the type series of *Aphis parvus* Theobald, which was described from chrysanthemums in Egypt but is currently placed as a synonym of *A. gossypii*.

Discussion

The results of the present study reveal the existence of a morphologically distinct and widely distributed form of *A. gossypii* colonizing plants of the family Compositae (Asteraceae–Cichoriaceae). The morphological separation of the Compositae-feeding form from aphids from Malvaceae and Cucurbitaceae did not involve the examination of single characters which is often ineffective and rarely found in the literature, but was based on CVA which compares the correlations between numerous characters. CVA has proved in earlier studies to be a powerful tool for resolving taxonomic problems among closely-related aphid taxa. When applied to aphid lineages reared under controlled conditions (Blackman & Spence, 1994), it separates environmental from genetic components of variance and can thus

Table 3. Fisher's linear discriminant functions (LDFs).

Characters	LDFs derived from the training subset		LDFs derived from the whole data set	
	Compositae	Non-Compositae	Compositae	Non-Compositae
urs	3770.8	3506.2	3863.2	3586.3
ht ii	1028.6	892.6	1344.8	1191.4
ant v	−241.3	−286.2	−430.0	−469.9
pt	215.0	258.1	249.9	293.3
lc	493.8	455.5	344.0	294.2
ls	−451.8	−346.7	−367.5	−298.3
mt	−254.5	−285.8	−16.4	2.8
ff	–	–	−333.0	−358.8
Constant	−193.9	−167.1	−198.6	−172.0

ant v, length of fifth antennal segment; ff, length of front femur; ht ii, length of second segment of hind tarsus; lc, length of cauda; ls, length of siphunculus; mt, length of middle tibia; pt, length of terminal process of sixth antennal segment; urs, length of ultimate rostral segment.

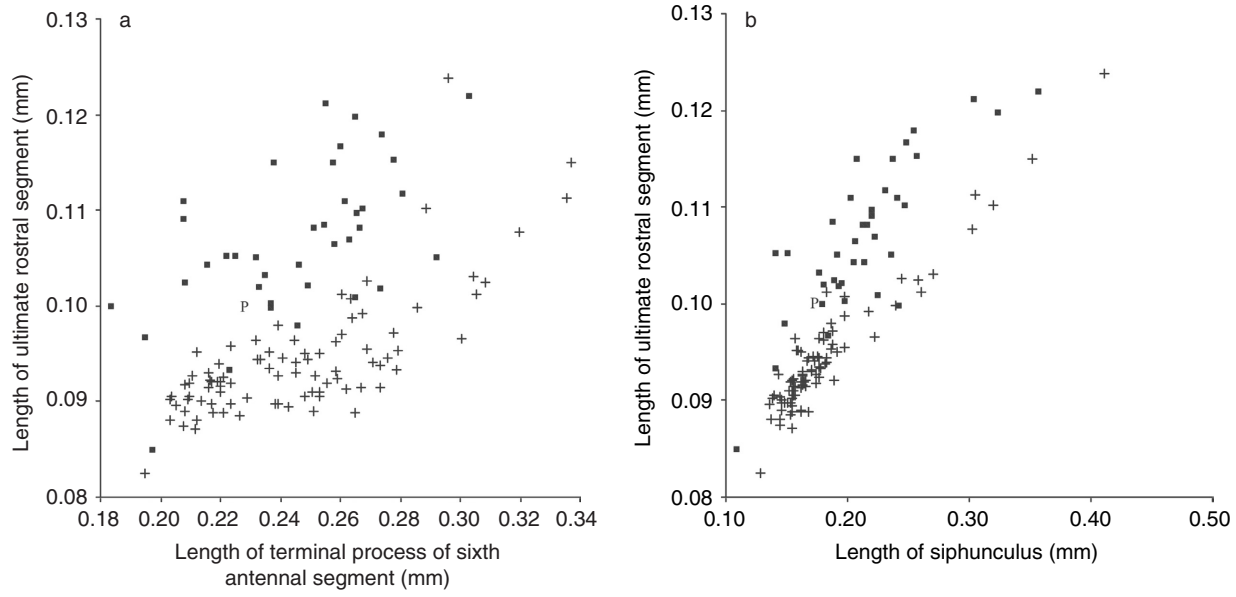

Fig. 6. Bivariate plots of the mean lengths in mm of (a) ultimate rostral segment vs. terminal process of sixth antennal segment and (b) ultimate rostral segment vs. siphunculus for laboratory-reared lineages and field samples of aphids from Compositae (■) and Cucurbitaceae–Malvaceae (+). P = one aptera from the type series of *Aphis parvus* Theobald (1915).

distinguish even between different genotypes. It can also be usefully applied to samples from field populations (Blackman & De Boise, 2002; see also the review by Blackman, 1992 for the properties of the method).

A criticism (e.g. Wool & Hales, 1997) often made of the use of multivariate analyses to describe taxonomic relationships within an insect species group is that the environmental component of variation may be large and as a consequence the morphological differences may not have a genetic basis. Temperature can affect both isometric and allometric growth in aphids and its interaction with genotype is complex (Blackman & Spence, 1994). Furthermore, the species and the physiological condition of the host plant can significantly affect aphid morphology (Moran, 1986; Wool & Hales, 1997; Dixon, 1998; Margaritopoulos *et al*., 2000). Phenotypic plasticity, i.e. strong interaction between environmental and genetic components of variation, has been well demonstrated, for example in a completely asexual aphid species *Myzus antirrhinii* (Macchiati) (Blackman, 1987; see also the discussion of Blackman & Brown, 1991). *Aphis gossypii* is also a phenotypically very plastic species (Rosenheim *et al*., 1994), and Wool & Hales (1997) found that host plants affected the morphology of samples examined from different localities of Australia much more strongly than any genetic differences among samples. The multivariate analysis performed in the present study identified some large environmental influences on morphology, but in most cases it was nevertheless possible to find evidence of clustering that reflected host-related genetic differences. This evidence is based on two considerations. Firstly, aphids from Greece were reared on the same host under constant conditions and therefore any possible environmental influence was excluded or at least minimized. Lineages retained the morphology associated with the host family on which they were collected even after long-term rearing on excised leaves of pepper. Secondly, the separation among field samples combined or not with laboratory-reared lineages was based on vectors (CV1 in fig. 2, and CV1 and CV3 in fig. 4) with a strong genetic component, i.e. those that did not show significant correlation with the size index, which has been shown to be strongly affected by environmental factors (Blackman & Spence, 1994).

To interpret taxonomic relationships within an aphid group based only on morphological variation may be a little risky. However, our study targets a species in which there is already evidence of intraspecific adaptation to host plants in terms of reproductive performance or related traits, i.e. on chrysanthemum (Europe: Furk & Hines, 1993; Guldemond *et al*., 1994), cucumber (Europe: Furk & Hines, 1993; Guldemond *et al*., 1994; China: Zhang & Zhong, 1990) and cotton (China: Zhang & Zhong, 1990; Australia: Wool & Hales, 1997), as well as variation in insecticide resistance related to host-plant preference (e.g. Furk *et al*., 1980; Furk & Vedjhi, 1990; Saito 1991; Furk & Hines, 1993). The results obtained here provide evidence of an association between host-plant and morphology, as found in other aphid species and species-groups, e.g. *Amphorophora* spp. (Blackman *et al*., 1977), *Rhopalosiphum maidis* Brown & Blackman (1988), *Myzus persicae* (Blackman, 1987; Margaritopoulos *et al*., 2000; Margaritopoulos *et al*., 2005) and *Euceraphis* spp. (Blackman & De Boise, 2002). However, it is unusual to find specificity at the level of the host family within what is regarded as one aphid species.

One contradictory point is that two *A. gossypii* samples from Brazil on *Carthamus tinctorius* (Compositae) did not behave consistently, and in one analysis clustered with aphids from Malvaceae and Cucurbitaceae. There seem to be several possible explanations. Firstly, it would be surprising if the host association of the two forms was a strict one, with the Compositae-adapted form never found on other hosts, and vice versa. Aphids introduced into new regions may show an expansion of host range, e.g. *Uroleucon ambrosiae*

(Thomas) (Hemiptera: Aphididae) in South America (Carvalho *et al*., 1998). Secondly, some genotypes of the Compositae-feeding form might have similar morphology to aphids colonizing other hosts. The number of different genotypes present in the dataset is unknown, and when CVA was applied to the samples from Compositae alone, most samples outside Europe were located away from the cluster of European samples, indicating that the degree of morphological divergence may be related to geographic separation and/or the length of time that populations have been apart. Wool & Hales (1997) examined Australian samples of *A. gossypii*, mainly from cotton and *Hibiscus* but also from sunflower *Helianthus annuus* L. (Compositae), *Clerodendron* sp. (Verbenaceae), *Lantana* sp. (Verbenaceae) and eggplant. They did not detect any morphological differentiation between aphids from sunflower and other hosts, and concluded that aphid morphology was affected more by the host plant on which they were reared than by any genetic differences among samples. It is likely that *A. gossypii* introduced into Australia has less genetic variation and perhaps does not include the Compositae-adapted form. This could also explain the anomalous result for the samples from *Carthamus tinctorius* in Brazil.

Two other anomalous samples were those from *Hibiscus syriacus* (Malvaceae) in Serbia and from *Senecio vulgaris* (Compositae) in Lebanon. These grouped together outside the main clusters in one analysis (fig. 3) or only the sample from Serbia in another analysis (fig. 4). On further examination, the slide-mounted specimens of both these samples were found to have morphological features associated with alatiformity, i.e. although unwinged they had some sclerotization of the thorax, a dark cauda and relatively long antennae. Alary polymorphism is a complicating factor in aphid morphology, as alate morphs not only differ from apterae by the presence of wings but also in many other morphological features. Development of alatae is induced under certain environmental conditions, and aphid colonies experiencing intermediate conditions at a critical stage of development may produce individuals that are intermediate between apterae and alatae. This intermediacy may have resulted in character correlations that placed their CV values together and outside the main clusters of true apterae.

No other clustering according to host plant was found except for that separating aphids from Compositae and non-Compositae. The aphids collected from Malvaceae and Cucurbitaceae in Greece, UK and Serbia (except the field sample from *Hibiscus syriacus* from Serbia referred to above) were located in a single cluster. This suggests that the same genotypes colonize both these plant families. Nevertheless, there are indications that other instances of host association within the non-Compositae group could be revealed by further work, particularly if samples from outside Europe were examined. For instance, Zhang & Zhong (1990) found in China that anholocyclic genotypes were associated with cucumber and holocyclic ones with cotton. Furthermore, Vanlerberghe-Masutti & Chavigny (1998) using the random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) method showed genetic differentiation between two samples from Malvaceae (cotton and *Hibiscus*) and samples from various cucurbits. Their material also included one sample from chrysanthemum, which, in contradiction to our findings, grouped with their two samples from Malvaceae. However, estimates of genetic affinity based on RAPD–PCR may not always be reliable (Isabel *et al*., 1999).

On a world scale, the life cycle variation and host relations of *A. gossypii* are so complex that further work will be necessary before any overall conclusions about the taxonomic status of the Compositae and non-Compositae forms are possible. The morphological similarity among some geographically-separated samples supports the suspicion that certain permanently parthenogenetic genotypes adapted to Compositae or Cucurbitaceae–Malvaceae are widely distributed, but this needs to be confirmed by molecular work, e.g. using microsatellite DNA analysis. There is also evidence of morphological divergence between geographically distant populations, e.g. between European and some non-European populations on Compositae (fig. 5). It is particularly necessary to investigate the situation in regions of East Asia and North America where some populations have a regular annual sexual phase on various woody hosts, and also to study further the relationships with other members of the *frangulae* group in Europe which utilize *Rhamnus* as primary host. The adaptation to Compositae presumably involved rather complex genetic changes requiring genetic recombination. It is possible that the Compositae and non-Compositae forms are associated with different primary hosts, and originated in different regions, parthenogenetic lineages of both then becoming dispersed to other parts of the world on their respective host plant families. The Compositae-feeding form has been shown capable of producing sexual morphs (Guldemond *et al*., 1994), but it has not yet been shown to have a regular annual sexual phase, and nothing is known about its primary host plant.

Some of the outliers in CV plots in the present paper could be other members of the *frangulae/gossypii* group, most of which are described as new on the basis of differences in their colour, host-preference or biology, and none of which can be reliably identified using morphological characters alone. The sample from *Ecballium elaterium* from central Greece, for example, an outlier in fig. 1, and also in the analysis examining only samples from Malvaceae and Cucurbitaceae, could possibly be *Aphis ecballii* Rusanova (Hemiptera: Aphididae), described from *Ecballium* in Azerbaijan (Rusanova, 1948), the description of which does not distinguish its apterae from those of *A. gossypii*.

The two forms identified in the present paper are presumably genetically isolated from one another to a large extent because their host-related morphologies are consistent over space and time. In particular, the Compositae-feeding form included samples collected over a 40-year period. The widespread prevalence of permanently parthenogenetic reproduction in *A. gossypii* may have assisted this isolation, limiting the opportunities for interbreeding. The two forms could be regarded as host races of *A. gossypii* (Drès & Mallet, 2002). Various authors (Müller, 1986; Rakauskas, 2004; Blackman & Eastop, 2006) have, however, argued that, in the case of pest species, it is important that intraspecific forms with consistent, recognizable and economically significant properties should be identified in the literature by formal, indexable Latin names, and that the subspecies category is appropriate for this purpose. We believe that subspecific names may eventually be useful in this case. However, in view of the apparent complexity of relationships within the *A. frangulae/gossypii* group, we suggest that further clarification of the relations between

the two forms, particularly with regard to primary host associations, is needed. For the present, we note that *A. gossypii* was originally described from cotton in North America (Glover, 1877), so the form on Cucurbitaceae–Malvaceae must be regarded as *A. gossypii* sensu stricto. If future work shows that a formal name is useful then the appropriate subspecies name for the Compositae-adapted form would be *A. gossypii parvus* Theobald. *Aphis parvus* was described from cultivated chrysanthemums in Egypt (Theobald, 1915), and an aptera from the type series in the NHM collection has the morphological characteristics of the Compositae-feeding form (fig. 6).

Measuring many characters on a series of specimens, incorporating them into an existing data base and performing CVA in order to establish the identity of new samples is a rather laborious procedure and may be considered impracticable. An attempt was made to develop linear discriminant functions (LDFs) for the identification of the Compositae-feeding form. The percent misclassification (12.7%), however, shows that these functions have some limitations. It is also possible that multiple-character LDFs will discriminate less satisfactorily when applied to new samples, as has been found in other aphid species (e.g. Blackman & Brown, 1991). It may be simpler and no less reliable to try to establish identity by measuring the three characters pt, urs and ls on new specimens and plotting their positions on the bivariate plots in fig. 6. With further work it may also be possible to produce LDFs and similar plots to aid in the identification of alatae, which are likely to show differences paralleling those found in apterae.

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