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Morphometric studies of the genus *Sitobion* Mordvilko 1914 in Australia (Hemiptera: Aphididae)

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Abstract The taxonomic status of several Australian populations within the aphid genus *Sitobion* has been uncertain for many years. Morphometric investigations using principal component analysis and canonical discriminant analysis have allowed us to clarify the relationships of these entities. A form on grasses, referred to in the literature as *Sitobion* near *fragariae*, is shown not to separate morphometrically from *S. fragariae* (Walker) collected in Europe and North America. In contrast, *Sitobion miscanthi* (Takahashi) Clones 61 (2n = 20) and 34 (2n = 17) separate unequivocally from each other and from Clone 4 (2n = 18), the supposed ancestral karyotype. We argue against describing these as separate species. Sexual forms of *S. miscanthi* and Australian *S. fragariae* reared in the laboratory were compared with the sexual forms of European *Sitobion avenae* (Fabricius) and *S. fragariae*, respectively. Australian specimens from *Smilax glyciphylla* and *Smilax australis* (Smilacaceae) are not *Sitobion smilacifoliae* (Takahashi).

Key words canonical discriminant analysis, chromosomal race, principal component analysis, sexual morph.

INTRODUCTION

The taxonomy of the Australian and Asian species of *Sitobion* Mordvilko (earlier considered a subgenus of *Macrosiphum* Passerini) on grasses is confused (Turak 1992; Blackman & Eastop 2000), with the possibility of synonymies, clines and primary host forms described under separate names. An aphid similar to the European species *Sitobion fragariae* (Walker) arrived in Australia and New Zealand probably some time in the 1960s, and has been the subject of papers by Hales and colleagues under the name *Sitobion* near *fragariae* (e.g. Sunnucks *et al*. 1996; Hales *et al*. 1998). M Carver (pers. comm. 1971) and VF Eastop (pers. comm. 1989) independently concluded from unpublished morphometric correlations that this species might be distinct from *S. fragariae* (Blackman & Eastop 2000). For example, VF Eastop (unpubl. data 1989) found that the ratio of body length or siphuncular length over caudal length for wingless specimens from Tasmania and New Zealand, probably *S*. nr *fragariae*, tended to be intermediate between that of *S. fragariae* and *Sitobion miscanthi* (Takahashi 1921). The ratio of siphuncular length to the length of the reticulated area of siphunculi in winged individuals was likewise intermediate. Furthermore, *S.* nr *fragariae* contains very few microsatellite alleles in common with *S. fragariae* from southern England (Sunnucks *et al*. 1996, 1997). VF Eastop (pers. comm. 1990) suggested that '*S.* near *fragariae*' might be *Sitobion kamtshaticum* (Mordvilko), possibly synonymous with *Sitobion rubiphilum* Takahashi, but could not obtain material to test this hypothesis. In both *S.* nr *fragariae* and *S. miscanthi* in Australia, genetic diversity assessed by microsatellites is extremely low (Sunnucks *et al*. 1996; Wilson *et al*. 1999). There is thus an intrataxon sampling problem in examining the Australian material. Despite this, significant differences were shown in host relations in collections from different locations, indicating differences in quantitative trait genes (Sunnucks *et al*. 1998). Two separate pieces of data suggest that *S. rubiphilum* is not conspecific with *S. fragariae*, although both items have the same sampling issue and are not robust. EF1α intron is different in a Japanese *S. rubiphilum* from that in *S*. nr *fragariae* and S. *fragariae* (Sunnucks *et al*. 2000 and unpubl. data 1998), and *S. rubiphilum* is different in mtDNA sequence and pseudogenes from *S.* nr *fragariae* and *S. fragariae* (Sunnucks & Hales 1996). *S. fragariae* is known from Europe, the Mediterranean, the Middle East, Pakistan and Nepal, and has been introduced to South Africa and to North and South America (Blackman & Eastop 2000).

It has been demonstrated that *S. miscanthi* in Australia and New Zealand is divided into closely related chromosomal races having all diploid numbers from 17 to 22, although the New Zealand 19 chromosome form was not definitely identified as a member of the *S. miscanthi* group (Hales *et al*. 1990; Sunnucks *et al*. 1996; Wilson *et al*. 1999; DF Hales unpubl. data 1998). There were two 2n = 18 karyotypes, one normal and one re-arranged. Qualitative observations suggested some differences in cuticular pigmentation and internal colour

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between 2n = 18 and 2n = 17 or 20 *S. miscanthi*, although the difference between the latter two was slight (Turak 1992). Choe *et al*. (2006) recently concluded, on the basis of morphometric and molecular evidence, that both *S. miscanthi* and the Asian *Sitobion akebiae* (Shinji) should be synonymised under *Sitobion avenae* (Fabricius), the now widespread European grain aphid. Both *S. fragariae* and *S. miscanthi* are minor vectors of barley yellow dwarf virus.

Additionally, *Sitobion* populations occur on *Smilax glyciphylla* and *Smilax australis* in Australia. Specimens were initially identified as *Sitobion smilacifoliae* (Takahashi): a specimen in the Natural History Museum, London (NHM), collected by the first author in 1970 and identified by Carver (RL Blackman pers. comm. 2007) provided the basis for inclusion of this species in the Australian Faunal Directory checklist (Hollis & Eastop 2005). We compare here Australian and Asian material with Taiwanese specimens of *S. smilacifoliae* collected and identified by Takahashi. The final representative of *Sitobion* in Australia is the yellow *Sitobion luteum* on orchids: there is no ambiguity about this species. The objective of this paper is thus to determine morphometrically the status of *S.* nr *fragariae*, Australian specimens on *Smilax* and chromosomal races of *S. miscanthi*.

Blackman and colleagues (Blackman *et al*. 1977, 1995; Blackman & Spence 1994; Blackman & De Boise 2002) have shown that canonical variates analysis may separate aphid species where simpler statistical methods do not. In order to carry out this analysis, one requires a large number of samples, each of five to 10 biologically coherent specimens (e.g. collected at the same time from a single host, or reared in the same conditions) and for each of the specimens a complete set of the chosen morphometric measurements needs to be recorded. We therefore applied this technique to available Australian *Sitobion* specimens from the *fragariae* and *miscanthi* groups. During his 1989 visit to Australia, Eastop reared and measured large numbers of *S.* nr *fragariae* and three chromosomal races of *S. miscanthi* at different temperatures, and the mounted specimens from these rearings provided the bulk of the material measured in the present investigation. The original specimens for these rearings were obtained from clones started from single wingless females and kept in culture by Hales. (Strictly speaking, these 'clones' should be called lineages, see Wilson *et al*. 2003.) Their origins are given below. They were supplemented with field-collected specimens from the Australian National Insect Collection (ANIC) and the NHM, including *S. fragariae* from both grasses and the primary host *Rubus*, as well as specimens of *Smilax* aphids from the collection of DFH, the NHM and Taiwan Agricultural Research Institute Insect Collection (TARI). The measurements included those that Eastop found most likely to separate *S.* nr *fragariae* from *S. fragariae*.

During earlier investigations (Wilson 2000; Wilson & Sunnucks 2006; DF Hales unpubl. data 1995), we reared sexual forms of *S. miscanthi* and *S.* nr *fragariae* in conditions of low temperature and long scotoperiod in the laboratory. They were compared in the present work with equivalent morphs of *S. avenae* and *S. fragariae*, respectively, from Europe. The sexuales of *S. miscanthi* have not previously been clearly associated with the parthenogenetic forms (see David 1975), and observations are briefly reported here.

MATERIALS AND METHODS

Material measured for statistical analysis

From NHM. *Sitobion fragariae* ex *Lolium perenne*, Ashurst, NZ, AGM Christensen, 7.xi.1983; swept, Picton, NZ, VFE 16.xii.1983; ex *Bromus* sp., Christchurch, NZ, AD Lowe, 2.xi.1976; ex *Bromus fanki*, Christchurch, NZ, VFE, 10.xii.1983; ex *Poa trivialis*, Kew Gardens, England, leg. SA Revoize, 21.vi.1972; ex *Rubus* spp., Lyndon, WA., USA, leg. RH Converse, 20.v.1971; ex *Rubus*, Harpenden, Herts, England, CTG, 31.v.1942; Malaise trap, Kifissia, Greece, LA Mound, 8.vi.1974; ex?*Festuca pratensis*, Petworth, Sussex, England, ex cult., 14.vii.1969; ex grass, Cap d'Antibes, France, leg. vs. d. Bosch, 8.v.1962; Exp Cage 3A, St Albans, HT, England, ix.1958; ex *Rubus* sp., Wye, Kent, England, CTG leg., 3.vi.39; ex *Dactylis glomerata*, Harpenden Common, Herts., HLW leg., 17.vii.1979; ex *Bromus arvensis* (thrashed) Rothamsted, Harpenden, Herts., England, 20.viii.1957; males flying, Bushy Park, Middx, 11.xii.1965, KL Harris; males suction trap, Silwood Park, Berks, 12–18.xi.1973; male and oviparae *Rubus fruticosus* group, Radcliffe-on-Trent, Notts, England, 15.xi.1980, JH Martin, oviparae *R. fruticosus*, BM(NH) front garden, Cromwell Road London, 10.xi.1978, GWW238. *Sitobion avenae* male, Suction trap, Elgin, Scotland, 16.x.1972, leg. Rothamsted; male, suction trap, Edinburgh, East Craigs, Scotland, 14.x.1972, leg. Rothamsted; males, Trap F6/43, Spalding, Lincs, 2.xi.1943, JP Doncaster; *Sitobion smilacifolium* [*sic*] ex *Smilax glabra* leafless tendrils, slopes of High West, Hong Kong Island, 28 November 1999, JH Martin; ex *Heterosmilax japonica* var. *gaudichaudiana*, Hong Kong, Hatton Road path, 1 December 2005, JH Martin; *S. smilacifoliae* ex *Smilax* sp. Thailand, Chiengmai Province Dai Suthep Sangival, 1150 m, 29.vi.1980, Dr H Bänziger; same data except 1480 m, 10.xii.1980.

From ANIC. Material of *S. miscanthi* Clone 4 (2n = 18) (collected Macquarie University 12.xii.1986, ex *Paspalum dilatatum*, DFH), Clone 61 (2n = 20) (collected Macquarie University, 9.iii.1988, ex *P. dilatatum*, DFH), Clone 34 (2n = 17) (collected Macquarie University, 3.xii.1986, ex *P. dilatatum*, DFH) and of *S.* nr *fragariae*, Clone 17 (obtained from PR Ridland, Plant Research Institute, Burnley, Vic., but originally collected in Tasmania), reared on barley or wheat for known numbers of generations at 10–12, 16–18, 22–24 or 26–29°C; ex oats, Toothdale, NSW, K Helms, M Carver, 14.viii.1985; green trap, Toothdale, NSW, April–November 1985; ex *Poa triviale*, Bennekom, Netherlands, leg. HRL, 17.xi.1946.

From TARI. Slide 31-5-3-5 ex *Smilax*, *Macrosiphum smilacifoliae* Takah. 2 October 1929, Suisha, R Takahashi; slide 31-5-3-1 ex *Smilax*, *M. smilacifoliae* Takah. 8 July 1934,

Table 1 Numbers of specimens and samples for males and sexual females (oviparae)

Sitobion	Males		Oviparae	
	No. specimens	No. samples	No. specimens	No. samples
Sitobion fragariae	6	3	8	2
Sitobion near *fragariae*	15	(Same lineage)	5	(Same lineage)
Sitobion miscanthi	12	3	10	3 lineages
Sitobion avenae	4	2	10	4 lineages

Sozan, R Takahashi; slide 31-5-3-2 ex *Smilax*, *Macrosiphum smilacifolium* [*sic*] Takah. 8.viii.1939, Domon, R Takahashi. **From Hales collection.** ex *S. glyciphylla*, West Pennant Hills NSW, 22.xi.1985, 4.xii 1985, Cumberland State Forest NSW 26–29.xi.1990, Thornleigh NSW 4.iv.2008, ex *S. australis*, North Stradbroke Island, Qld, 14.vi.2009, all collected by DFH. Laboratory-reared oviparous females and males of *S.* nr *fragariae* (Clone 17) and *S. miscanthi* (Clones 4, 195, 197, all 2n = 18), and oviparous females of *S. avenae* kindly provided by the laboratory of Dr J-C Simon, INRA, Le Rheu, France.

Methods

A total of 184 winged and 222 wingless specimens of the *S. miscanthi/fragariae* group were measured, with at least four specimens in each biologically coherent sample. The number of specimens of sexual morphs is given in Table 1. Measurements of slide-mounted specimens were taken using Miyazaki (1987) and Ilharco and van Harten (1987) as a guide for measuring morphological characters, except that body length was measured from the tip of the antennal tubercle to the end of the anal plate (VF Eastop, pers. comm. 2007). Phase contrast and differential interference microscopy were used as appropriate.

Measurements were taken for all specimens of lengths as in Table 2. The following set of measurements was added for winged individuals from Gramineae: length of longest seta on antennal segment 3, median dorsal cephalic seta, seta on abdominal tergite 8, longest seta on hind tibia, hind empodial seta and longest seta on hind second tarsal segment. These measurements did not improve separation and are not discussed further. For sexual females (oviparae), the number of scent plaques (pheromone glands) on each hind tibia was recorded. Measurements for males were as for winged parthenogenetic females; numbers of rhinaria on antennal segments 4 and 5 were also recorded.

Patterns of morphometric variation were analysed using two multivariate statistical approaches (Tabachnick & Fidell 2006). Principal component analysis (PCA; SAS procedure PRINCOMP, SAS version 9.1.3, SAS Institute Inc., Cary, NC, USA), 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, then iteratively calculates new combinations to explain any residual variation. This procedure does not assume any *a priori* groupings. Canonical discriminant analysis (CDA; SAS procedure CANDISC) operates on the mean values for groups defined prior to analysis, effectively providing linear combinations of variables that best summarise differences between classes. Discriminant function analysis (DFA) was used to determine potential misclassification of specimens.

Table 2 Variables measured

Variable description	Variable name
Body length	BL
Hind tibia length	hTib
Hind tarsus segment 2 length	hTars2
Length of antennal segments 3, 4 and 5	A3, A4, A5
Length of base of antennal segment 6	A6b
Length of processus terminalis of antennal segment 6	A6pt
Length of rostral segments IV + V	URSL
Siphuncular length	Siph
Cauda length	Cd
Length of reticulated area on siphunculi	siphRet
Additional variables for sexual forms	
Number of pheromone plaques on hind tibia of ovipara	pheT
Number of sensoria on antennal segments 3, 4 and 5 of male	A3rh, A4rh, A5rh

Analyses were carried out separately for wingless and winged parthenogenetic specimens. A combined analysis of all specimens of *S. fragariae*, *S.* nr *fragariae* and *S. miscanthi* was followed by separate analyses of *S. fragariae* + *S.* nr *fragariae* and of the three *S. miscanthi* clones. The males and oviparous females of *S. fragariae*, *S.* nr *fragariae*, *S. miscanthi* and *S. avenae* were analysed by PCA and CDA, but numbers were small (Table 1).

RESULTS

Results for common analysis of all samples of *S. miscanthi*/*S. fragariae* group

Preliminary examination of the correlation matrix of all variables showed that length of the hind tibia was correlated ($r = 0.97$) with length of antennal segment 3 and it was thus deleted in subsequent analyses as redundant. Variation in degree of abdominal segment telescoping may have rendered some body length measurements inaccurate; body length was thus excluded in calculation of CDA in some cases. In preliminary analysis, specimens of *S. fragariae* from the primary host,

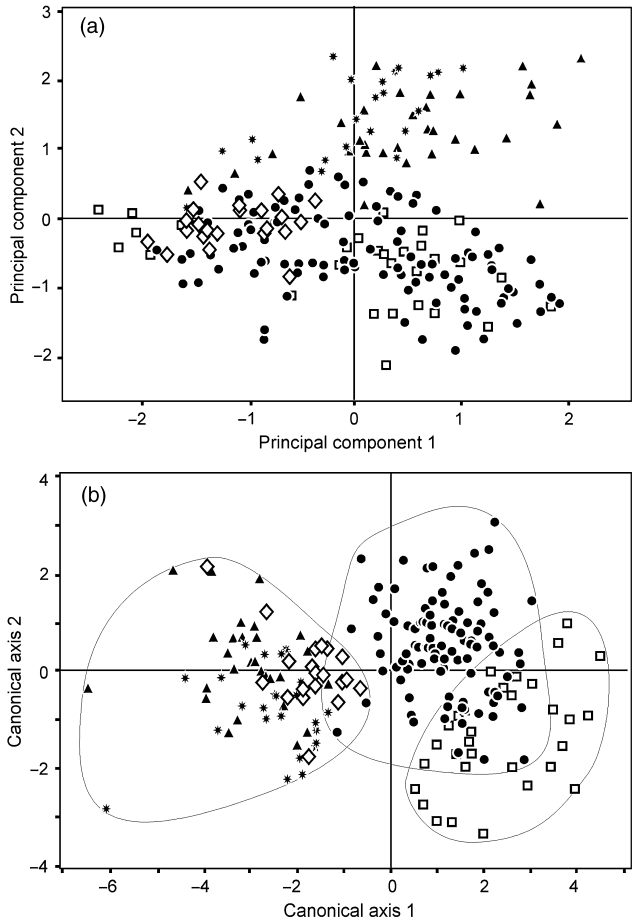

Fig. 1. Ordinations of apterous specimens of *Sitobion fragariae* on grasses, *Sitobion* near *fragariae* and *Sitobion miscanthi*. (a) Principal component analysis, ordination of apterous specimens on first two principal component axes. (b) Canonical discriminant analysis of three classes (*fragariae*, nr *fragariae* and *miscanthi*), ordination of apterous specimens on canonical axes. Note that although *S. miscanthi* specimens are labelled by clone in canonical discriminant analysis, they were combined as a single class in the analysis. Bounding lines are arbitrary group envelopes. Symbols: *S. fragariae* (□), *S.* nr *fragariae* (●), *S. miscanth*i Clone 4 (◇), Clone 34 (*), Clone 61 (▲).

Rubus, were clearly differentiated from the grass feeding forms. As primary host forms do not occur in Australia they are not considered further.

Wingless parthenogenetic females

An overall PCA showed that there are differences in character distribution among groups within the common components of total variation (Fig. 1a). The contribution of all variables to principal component 1 was positive, indicating that this axis reflects a general size component (confirmed by a linear relationship between body size and component 1, not shown). Position of specimens on the second component is largely due to length of the terminal process of the antenna, other distal flagellar segments, ultimate rostral segment (URS) and extent of siphuncular reticulation contrasted with lengths of other appendages, including siphunculus and cauda (contributions of variables to the first three components given in Table 3A). *S. fragariae* and *S.* nr *fragariae* exhibit similar patterns of variation. However, distribution along component 2 with increase in value of component 1 differs between *S. miscanthi* and *S. fragariae* + *S.* nr *fragariae* – 'smaller' specimens of both groups occupy similar space, but 'larger' *S. miscanthi* specimens have increasing values along component 2, whereas the values of component 2 for specimens of *S. fragariae* + *S.* nr *fragariae* are somewhat decreasing as size increases. In CDA with prior determinations (*S. miscanthi*, *S. fragariae* and *S.* nr *fragariae*) as the classification variable, *S. miscanthi* is largely separate from the other two groups (Fig. 1b). The analysis was able to extract some difference between the group means for *S. fragariae* and *S.* nr *fragariae*, but there is broad overlap among specimens. The main contribution to discrimination of *S. miscanthi* from the other groups is length of siphunculus and third antennal segment, in contrast to the length of the cauda and of fourth antennal segment (Table 3).

Winged parthenogenetic females

Results of PCA (Fig. 2a, Table 3B) of winged individuals of all samples show a pattern very similar to that shown by wingless females. Results of CDA (Fig. 2b, Table 4) are also similar to results for wingless females, i.e. *S. miscanthi* generally is well separated from *S. fragariae* + nr *fragariae*, whereas *S. fragariae* and *S.* nr *fragariae* show considerable overlap.

Comparison of *S. fragariae* and *S.* nr *fragariae*

Wingless parthenogenetic females

The PCA on specimens of *S. fragariae* and *S.* nr *fragariae* (Fig. 3a) indicates that there is large overlap in morphological variation in the variables measured for these two groups. One of the characters found to be intermediate between these groups by Eastop (relatively longer cauda in the latter) was assessed separately (Fig. 3b). Although the relative mean length of the cauda is longer in *S.* nr *fragariae*, as noted by Eastop, the range of variation in this character exhibited by *S.* nr *fragariae* encompasses the total range shown by the specimens of *S. fragariae* examined.

Winged parthenogenetic females

Results for winged females are similar (Table 4B).

Comparison of *S. miscanthi* clones

Wingless parthenogenetic females

In PCA of wingless females of *S. miscanthi*, the position of Clone 4 specimens clearly differs from that of the other two clones (Fig. 4a), whereas the distribution of samples of Clones 34 and 61 is less clearly different. However, CDA (Fig. 4b) is able to provide good separation of the latter two groups, with little overlap. The primary contributing factor in this discrimi-

Table 3 Proportion of variation and variable coefficients of first three eigenvectors (principal components) for principal component analysis of pooled samples of *Sitobion miscanthi*, *Sitobion fragariae* and *Sitobion* near *fragariae*, and total sample standardised canonical coefficients for canonical discriminant analysis with the three taxa as classification values

	Principal component 1	Principal component 2	Principal component 3	Canonical axis 1	Canonical axis 2
A – wingless females					
Proportion of total variation	73%	10%	5%		
bl	0.280	–0.373	–0.313	–	–
htib	0.326	–0.052	–0.063	–0.532	–0.621
cauda	0.287	–0.146	0.262	–1.041	1.054
siph	0.284	–0.400	0.097	2.578	1.476
siphret	0.254	0.100	0.750	–0.057	–0.502
A3	0.317	–0.011	0.027	0.744	–1.097
A4	0.316	0.217	0.018	–1.638	0.799
A5	0.315	0.171	–0.105	–0.161	0.455
pt	0.222	0.658	0.013	–0.399	0.716
A6b	0.291	0.142	–0.210	0.313	–1.186
ursl	0.273	0.149	–0.445	–0.029	–0.624
htars2	0.282	–0.341	0.047	0.539	–0.199
B – winged females.					
Proportion of total variation	69%	11%	5%		
bl	0.307	–0.193	–0.199	–	–
htib	0.332	–0.104	0.001	0.128	–0.196
cauda	0.291	–0.268	0.022	–0.522	–0.190
siph	0.292	–0.083	0.083	3.372	0.216
siphret	0.240	0.057	0.849	–0.344	–0.704
A3	0.326	–0.028	–0.043	–0.051	–0.022
A4	0.318	0.167	0.035	–1.101	–0.176
A5	0.313	0.171	–0.096	–0.380	–0.538
pt	0.198	0.660	0.137	–0.571	–0.114
A6b	0.272	0.351	–0.294	0.028	0.642
ursl	0.268	0.057	–0.338	–0.073	1.386
htars2	0.277	–0.306	0.008	0.257	0.157

nation is the contrast of length of antennal segment 3 with segment 4 and the cauda (Table 5A).

Winged parthenogenetic females

Winged specimens of the three clones are less clearly differentiated than are the wingless individuals (PCA, Fig. 5a). However, specimens of Clone 4 are largely positioned away from the other two clones, although some specimens of this clone are distributed throughout the range of common variation. CDA (Fig. 5b) yields less distinct separation of winged females of the three clones than is obtained for wingless females, and DFA cross-validation indicates that a relatively high number of specimens are misclassified (Table 5B).

Sexual forms of *S. miscanthi* and *S.* nr *fragariae*

All specimens of sexuals of *S. miscanthi* and *S.* nr *fragariae* and some *S. avenae* are laboratory reared, whereas those of *S. fragariae* and remaining *S. avena*e are field collected.

Principal component analysis of oviparous females

Contributions of variables to the first three principal components are given in Table 6A. The first component (52% of total variation) captures in general size variation. The second component (18% of the total variation) expresses the combination of length of siphunculus, distal antennal segments and number of pheromone glands contrasted with extent of siphuncular reticulaton and caudal length. It captures differences among *S. fragariae*, *S. miscanthi* + *S.* nr *fragariae* and *S. avenae.* The third component (9% of total variation) expresses the combination of number of pheromone glands, tarsal length and A6b, contrasted with length of A6pt, cauda, siphunculus and captures differences between *S. fragariae* + *S. avenae* and *S. miscanthi* + *S.* nr *fragariae*. The similar response of *S. miscanthi* and *S*. nr *fragariae* suggest that much of the variation is a reflection of the effects of rearing history.

Canonical discriminant analysis of oviparous females

The four groups are clearly separated by CDA (Fig. 6, Table 6A). Canonical axis 1 (accounting for 72% of variation among means) separates *S. avenae* from the others based mainly on siphunculus length, contrasting with hind leg (*htib* and *htars2*). Canonical axis 2 (21%) separates *S. miscanthi* from *S. fragariae* + *S.* nr *fragariae* based on number of pheromone glands along with hind leg and siphunculus length, contrasted with length of distal antennal segments and cauda. Axis

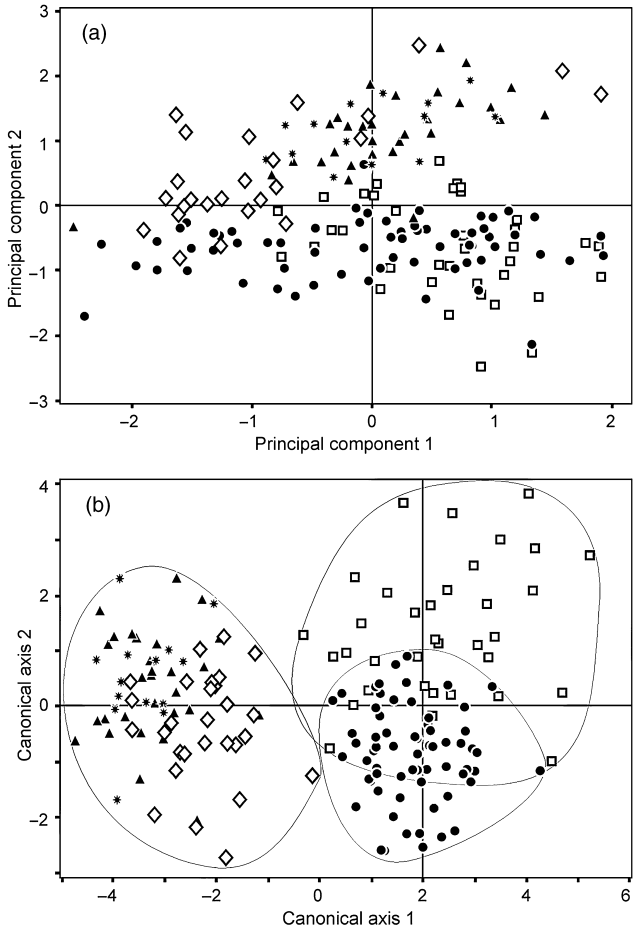

Fig. 2. Ordinations of alate specimens of *Sitobion fragariae* on grasses, *Sitobion* near *fragariae* and *Sitobion miscanthi*. (a) Principal component analysis, ordination of alate specimens on first two principal component axes. (b) Canonical discriminant analysis of three classes, ordination of alate specimens on first two canonical axes. Note that although *S. miscanthi* specimens are labelled by clone in canonical discriminant analysis, they were combined as a single class in the analysis. Bounding lines are arbitrary group envelopes. Symbols: *S. fragariae* (□), *S.* nr *fragariae* (●), *S. miscanthi* Clone 4 (◇), Clone 34 (*), Clone 61 (▲).

3 (7%) separates *S. fragariae* from *S.* nr *fragariae* based on length of A3 contrasted with length of siphunculus and hind tibia (Fig. 6b).

Sitobion near *fragariae* oviparae

Laboratory-reared oviparae of *S.* nr *fragariae* tended to have smaller numbers of pheromone glands on the hind femora than European *S. fragariae* (mean 157 vs. 241), lower Siph/Cd ratios (range 2–2.4 vs. 2.2–2.7, $n = 5$ and $n = 8$), and four Australian specimens had one or two sensoria on antennal segment 3 whereas no European specimens had any sensoria. The number of setae on the genital plate was lower in Australian specimens (range 16–21 cf. 22–36).

Description of *S. miscanthi* oviparae

Colour of living specimens. Straw-coloured; older specimens greenish to dark green. Eggs appear green through body wall. Eyes dark red-brown. Antennae black, A1 and 2 less densely so. Coxae and trochanters pale, femora dark pigmented for approximately distal two-thirds. Tibiae dark at femoral joint and distal quarter. Tarsi black. Siphunculi black, cauda pale, anal plate and anterior third of genital plate lightly pigmented.

Pigmentation in macerated specimens. Generally more intense than in *S. fragariae*; siphunculi black, distal 33–50% of femora black, distal 22–25% of tibiae black, tarsi black, last two rostral segments lightly pigmented, head lightly pigmented, antennae black, but lighter on antennal segments 1 and 2 (A1 and 2). Faint dorsal abdominal pigmentation. Paired small lightly pigmented plaques on abdominal tergites 1–6, the latter slightly mesad of the siphunculi. Cauda, anal and genital plates slightly more pigmented than general abdominal surface.

Morphology. Body length 1.57–1.88 mm. Antennae 1.36–2.02 mm, between 0.9–1.22 times body length, processus terminalis 1.2–1.7 times A3 and 4.5–6 times A6B. A1–2 smooth, distal part of antennal segments 3–6 increasingly imbricated. Antennal segment 3 with 0 or 1 medium to small rounded sensorium on the lateral surface. Tip of URS (= rostral segments IV + V) reaching mesothoracic trochanters. URS 0.84–1.01 times hind tarsal segment 2 and 0.76–0.99 A6B. Spiracles round. Siphunculi 0.3–0.48 mm in length, with reticulations on the terminal 13–22% and imbrications on the remainder; siphunculi 1.5–2.5 times the length of the cauda and 0.21–0.29 of the body length. Minimum width at the reticulated area 0.76–1.36 times the minimum thickness of the hind tibia. Cauda tapering to rounded tip, usually without evident constriction. Imbricated, with seven to 11 setae. Furca with a short stalk.

Chaetotaxy. Four setae on each of abdominal tergites VII and VIII, longest seta on A3 8–12 μm, much less than the diameter of the base of A3 (18–26 μm), longest dorsal cephalic seta 8–14 μm. URS with one pair of small anterior secondary setae, three pairs of primary setae (40 μm), two pairs of posterior secondary setae to 24 μm. First tarsal chaetotaxy 3-3-3. Dorsal setae blunt or pointed, anterior abdominal segments generally with one pair spinal, one pair pleural and three pairs of marginal setae to about 24 μm. Head dorsally and ventrally with four pairs of setae to 24 μm. Genital plate with up to 18 posterior marginal setae, up to six anterior setae and two primary setae. Anal plate with nine long setae to 62 μm. Caudal setae to 62 μm.

Laboratory-reared oviparae of *S. miscanthi* when compared with European *S. avenae* had much smaller numbers of tibial pheromone plaques (range 28–96 cf. 117–222). *S. miscanthi* had a maximum of 26 setae on the genital plate whereas *S. avenae* had up to 38.

Comments. The ovipara is readily distinguished from the viviparous female by the presence of scent plaques on the hind tibiae, which are concomitantly swollen. The series shows this

Table 4 Proportion of contribution and variable coefficients of first three eigenvectors for principal component analysis of pooled of *Sitobion fragariae* and *Sitobion* near *fragariae*, and total sample standardised canonical coefficients for canonical discriminant analysis with the two putative groups as classification values

	Principal component 1	Principal component 2	Principal component 3	Canonical axis 1
A – apterae				
Proportion of total variation	78%	6%	5%	
bl	0.287	–0.392	–0.170	0.033
htib	0.318	–0.080	0.105	–0.877
cauda	0.281	0.106	–0.459	1.739
siph	0.312	0.041	–0.129	–0.641
siphret	0.243	0.620	–0.292	–0.489
A3	0.311	–0.040	–0.045	–1.045
A4	0.314	0.075	0.020	1.456
A5	0.306	–0.069	0.152	0.695
pt	0.237	0.466	0.649	0.975
A6b	0.279	–0.123	0.316	–1.160
ursl	0.263	–0.444	0.189	0.009
htars2	0.297	–0.016	–0.253	–0.452
B – alatae				
Proportion of total variation	75%	7%	6%	
bl	0.295	–0.268	–0.110	–0.240
htib	0.323	0.006	–0.027	–0.160
cauda	0.286	–0.064	–0.515	–0.556
siph	0.317	0.093	–0.179	–0.642
siphret	0.204	0.776	–0.208	0.175
A3	0.318	–0.067	–0.038	0.250
A4	0.315	0.065	0.163	–0.869
A5	0.308	–0.042	0.273	0.653
pt	0.250	0.326	0.446	0.264
A6b	0.263	–0.249	0.520	1.454
ursl	0.263	–0.365	–0.271	0.022
htars2	0.298	0.006	–0.042	–0.240

character to varying degrees and the specimens appear to be intermediate in morphology between parthenogenetic females and true oviparae.

Males

Principal component analysis of males

Cauda, body length and A6pt were omitted from these analyses because of missing values. Contributions of variables to the first three components are given in Table 6B.

The first component (47% of total variation) indicates differences related to generalised size, with *S. miscanthi* specimens falling in the smaller range. The second component (16% of total variation) contrasts the number of rhinaria on A4 and length of hind tibia and siphunuculus with number of rhinaria on A3 and A5 and the length of A6b and hind tarsus, reflecting differences between S. *avenae* and all others.

Canonical discriminant analysis of males

The three groups *S. miscanthi*, *S. avenae* and *S. fragariae* + *S.* nr *fragariae* are clearly differentiated by the first two canonical axes (Fig. 6c), which together account for 87% of the variation among group means. The remaining variation (Fig. 6d, axis 3) separates *S. fragariae* from *S.* nr *fragariae* on length of A3 and A6b in contrast to length of siphunculus.

Description of *S. miscanthi* males

Colour of living specimens. Head and prothorax dorsally mid-brown, darker around ocelli. Eyes deep red. Antennae black, though segments 1–2 not densely so. Rostral segments III–V blackish. Meso- and metathorax dorsally dark brown at muscle insertions, mid-brown elsewhere. Coxae and trochanters pale. Femora pale proximally and black distally; one-third to one-half black on prothoracic leg and one-half to two-thirds on the other legs. Tibiae black at femoral articulation and distal fifth to quarter. Tarsi black. Abdomen greenish or brownish (in younger specimens) with incomplete black bars in some specimens. Siphunculi black, cauda, anal plate and aedeagus pale, claspers black. Immature stages pink.

Pigmentation of macerated specimen. Head, antennae, thorax and siphunculi pigmented, legs pigmented ranging to black on distal third of fore-femora and two-thirds of mid- and hind femora, and on distal fifth of tibiae, tarsi pigmented, rostral segments IV–V lightly pigmented, broken bands of pigmentation on abdominal terga 2–5, pigmentation behind siphunculi and across terga 7–8. Lateral pigmented patches on

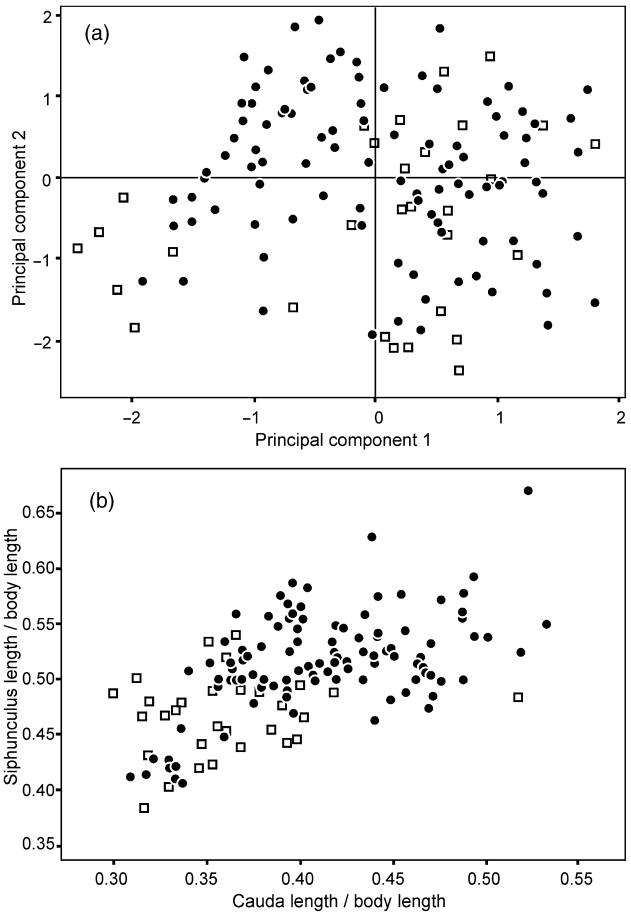

Fig. 3. Ordination of apterous specimens of *Sitobion fragariae* (□) and *Sitobion* near *fragariae* (●). (a) Principal component analysis, ordination on first two component axes. (b) Caudal length vs. length of siphunculus, normalised by body length.

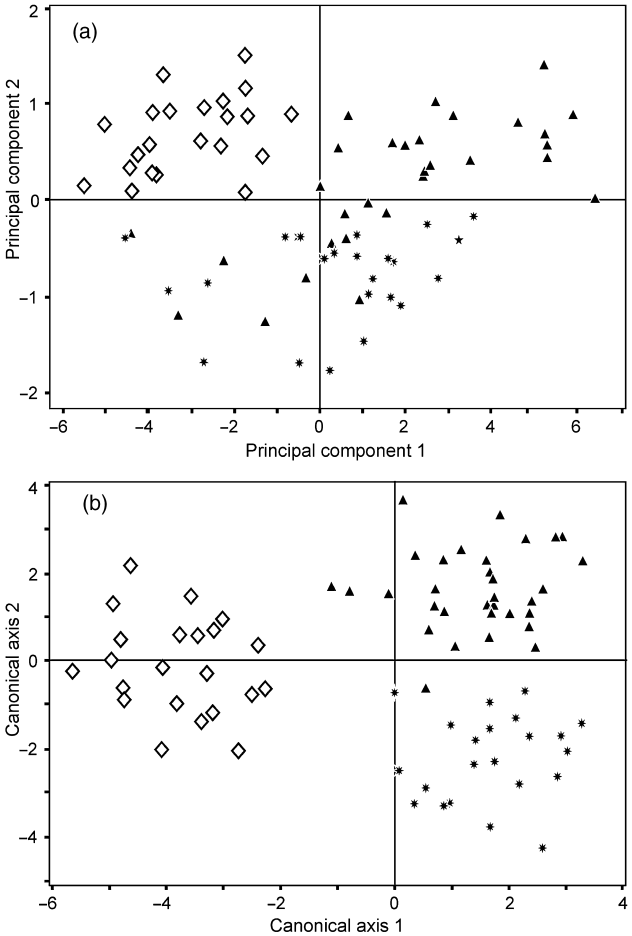

Fig. 4. Ordination of apterous specimens of *Sitobion miscanthi* clones. (a) Principal component analysis, ordination on first two component axes. (b) Canonical discriminant analysis, ordination on first two canonical axes. Points labelled according to clone: Clone 4 (◇), Clone 34 (*), Clone 61 (▲).

abdominal segments 2–4. Claspers pigmented. Anal plate slightly more pigmented than general abdominal surface.

Morphology. In this series, all specimens had shrunken abdomens so that body length could not be measured. Antennae 1.92–2.96 times length of hind tibia, processus terminalis long, 1.43–1.73 times A3, A1–2 smooth, A3–6 increasingly imbricated. A3 with 41–62 sensoria (mean 52.3), A4 with 17–23 (mean 19.4) and A5 with 11–20 (mean 14.3). Rostral segments IV–V 0.74–1.0 times hTars2. Siphunculi without marked swellings or constrictions, with reticulation on the terminal 23–36% and imbrications on the remainder; a small terminal flange. Cauda broadly conical, imbricated, with five to six setae. Siphunculi 0.23–0.33 mm, 2.1–2.8 times the length of the cauda.

Chaetotaxy. Dorsal setae pointed, 18–26 µm, ventral setae pointed, to 30 µm, A3 setae 12–16 µm, 0.40–0.62 times base of A3. URS bears three pairs of primary setae, the longest being laterally placed and about 44 µm in length. Its anterior surface has two pairs of secondary setae, about one-quarter and one-half the distance from the basal articulation and about 20 and 35 µm long, respectively, whereas the posterior surface has a single pair of secondary setae about 18 µm in length. First tarsal chaetotaxy 3-3-3. Cauda with three to 11 setae to 66 µm in length. Claspers with about 24 setae on the sclerotised surface and eight on the inner surface.

Comparison of male and ovipara with those of *S. avenae* and previously observed possible *S. miscanthi* sexuals

Ghosh and Ray Chaudhuri (1962) described oviparae as *M.* (*S.*) *fragariae* and subsequently Ghosh *et al.* (1972) stated these specimens to be *S. miscanthi*. They recorded two similar but larger oviparae in association with viviparous females of *S. miscanthi* on unidentified grass in West Bengal, as well as four male *Sitobion* sp. that could not be assigned to species. On re-examination, Raychaudhuri (1980) concluded that the oviparae were not *S. miscanthi*. The males had A6pt/A3 of 1.2, Siph/Cd of 2.3, and sensoria distributed 48–62, 32–41 and 24–25 on A3–5. Our laboratory-reared *S. miscanthi*, for comparison, had A6pt/A3 1.4–1.7, Siph/Cd 2.1–2.8, and sensoria

Table 5 Proportion of contribution and variable coefficients of first three eigenvectors for principal component analysis of *Sitobion miscanthi* lineages, and total sample standardised canonical coefficients for canonical discriminant analysis with the three lineages as classification values

	Principal component 1	Principal component 2	Principal component 3	Canonical axis 1	Canonical axis 2
A – apterae					
Proportion of total variation	79%	6%	4%		
Bl	0.327	0.016	0.093	–	–
Htib	0.279	0.443	0.207	–2.705	–3.071
cauda	0.314	0.146	0.185	–0.730	1.434
Siph	0.268	–0.009	0.736	–0.294	0.430
siphret	0.319	–0.217	0.144	–0.098	0.015
A3	0.326	–0.168	–0.087	3.135	–1.920
A4	0.325	0.013	–0.204	1.877	2.159
A5	0.303	–0.361	–0.243	–0.531	1.635
Pt	0.306	0.025	–0.202	–0.538	0.339
A6b	0.281	–0.406	–0.201	1.021	–0.722
Ursl	0.256	0.641	–0.403	0.849	0.291
htars2	0.327	0.016	0.093	–0.030	0.392
B – alatae					
Proportion of total variation	76%	6%	6%		
bl	0.297	0.136	0.081	–0.094	0.495
htib	0.319	–0.102	–0.006	0.065	0.836
cauda	0.263	0.129	0.565	0.107	0.331
siph	0.316	–0.109	–0.109	0.132	0.499
siphret	0.279	0.205	0.146	–0.133	0.422
A3	0.309	–0.269	0.008	0.146	0.455
A4	0.310	–0.008	–0.141	0.294	0.386
A5	0.310	–0.123	–0.283	–0.049	0.559
pt	0.285	–0.273	0.017	0.290	0.328
A6b	0.291	–0.256	–0.333	–0.043	0.708
ursl	0.262	0.203	0.496	0.426	0.212
htars2	0.200	0.795	–0.429	–0.094	0.495

distributed on A3 41–62, on A4 17–23 and on A5 11–20. David (1975) also referred to males unassociated with other morphs or usual host plants.

Comments

The oviparae of *S. miscanthi* available for this analysis were distinguishable from those of *S. avenae* by use of the multivariate methods above. *S. miscanthi* had a greater ratio of rostral segments IV–V/hTars2, Siph/BL, Siph/C and A6pt/b. *S. miscanthi* had a smaller proportion of reticulation on the siphunculi, a smaller number of pheromone plaques and shorter setae on antennal segment 3. In addition, macerated *S. avenae* displayed distinctly pigmented coxae and genital plate, whereas these structures were barely pigmented in *S. miscanthi* oviparae. The laboratory-reared *S. miscanthi* oviparae, although functional as mating, egg-laying females, showed features tending towards the viviparous facies. Yet-to-be-found Asian field-collected specimens may have, e.g. greater numbers of pheromone plaques, shorter, stouter hind tibiae and shorter antennae than those examined here. The fact that functional sexual forms could be reared in the laboratory strongly suggests that sexual forms may occur naturally in Asia.

Males of *S. miscanthi* have a relatively shorter cauda and hind tarsal segment 2 than males of *S. avenae*, and a longer processus terminalis. They tend to have fewer sensoria on antennal segment 3 and more on A4 than *S. avenae*.

Comparison of specimens from Smilacaceae

Visual comparison of specimens from *Smilax* indicated that Australian material was quite different from *S. smilacifoliae* collected in Taiwan by Takahashi. Specimens from Hong Kong and Thailand conformed more closely to the Australian material than to the Taiwanese.

DISCUSSION

Sitobion nr *fragariae*

Multivariate analysis did not separate Australian and New Zealand *fragariae*-like forms from grass-feeding (secondary host) *S. fragariae* collected in the Northern Hemisphere, and therefore, despite the apparent differences detected by Carver and by Eastop, the Australasian specimens are unlikely to represent a distinct species. Australian specimens, collected

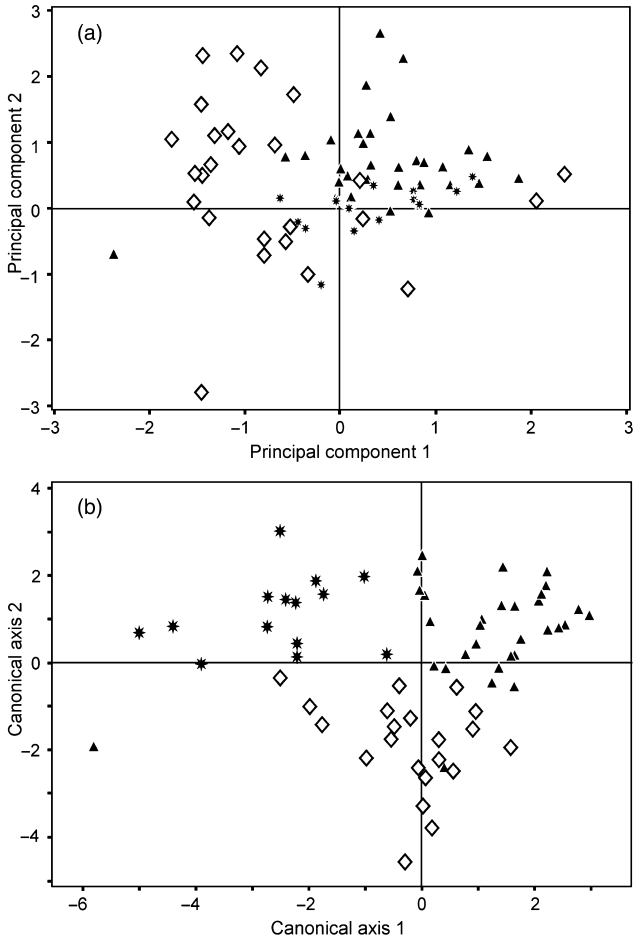

Fig. 5. Ordination of alate specimens of *Sitobion miscanthi* clones. (a) Principal component analysis, ordination on first two component axes. (b) Canonical discriminant analysis, ordination on first two canonical axes. Points labelled according to clone: Clone 4 (◇), Clone 34 (*), Clone 61 (▲).

from all states except the Northern Territory, were determined by molecular techniques to belong to the same clone (Sunnucks *et al*. 1996). The majority of '*S.* nr *fragariae*' specimens measured in this analysis were laboratory-reared members of the functionally parthenogenetic Australian clone, and were derived in the laboratory from one initial female for each of the two lineages represented. This might be considered a fault in procedure, but is mitigated by the wide range of temperatures under which the specimens were reared. Although growth in controlled conditions on barley seedlings is probably suboptimal for grass and cereal *Sitobion* species, it was observed that the maximum size obtained in culture was the same as the maximum size of both field-collected '*S.* nr *fragariae*' and *S. fragariae*, and that the minimum size was higher than that of field-collected *S. fragariae* from cereals and grasses, and only slightly less than field-collected '*S.* nr *fragariae*', as measured by hind tibia length in wingless females.

It seems likely that the trend towards differences between Australian and Northern Hemisphere *S. fragariae* represents a founder effect and subsequent evolution, with a single successful colonisation of Australia in the 1950s or 1960s. Host relations seem to have been subject to slight but measurable divergence in monophyletic, apomictic lineages within Australia of both *S. miscanthi* and *S.* nr *fragariae* (Sunnucks *et al*. 1998). New Zealand specimens included three microsatellite genotypes: the Australian genotype and two others that are consistent with being derived by mutation alone from a recent common ancestor shared with the Australian clone, one of which (Snf.NZ1) is by far the most common genotype in New Zealand (Wilson *et al*. 1999). Analysis did not distinguish field-collected New Zealand from Australian specimens although the most common genotype in the late 1990s was distinct from the Australian genotype. There is, however, no information on the history of this genotype in New Zealand: it may be a more recent arrival not represented in the older field-collected material available. No biological peculiarities have been noted in the Australasian *S. fragariae* to warrant recognition of a separate species.

Sitobion from *Smilax*

The Australian, Hong Kong and Thailand specimens are not *S. smilacifoliae* or other *Sitobion* species described from Smilacaceae in Asia. Further work is being done on this group and will be published separately.

Sitobion miscanthi and its chromosomal races

The distinct separation of the clones representing *S. miscanthi* chromosomal races is an unexpected result and gives rise to questions on their taxonomic status. Those with re-arranged karyotypes (Clones 61 and 34) share allele sizes at many loci with Asian specimens of *S. miscanthi* (Taiwan, see Wilson *et al*. 1999; Beijing area, Wang *et al*. 2008) and their identity as *S. miscanthi* is unequivocal. It is clear from previous work that they are obligately parthenogenetic lineages, whereas the 2n = 18 clone with an 'ancestral' karyotype is functionally parthenogenetic in Australia, but can be forced to reproduce sexually in the laboratory, and may be a member of a lineage doing so naturally in Asia. Following the arguments of Blackman and Brown (1991), a continuously parthenogenetic lineage of aphids arising from a bisexual ancestor is reproductively isolated and it may be appropriate to describe it as a separate species provided that it is morphologically or biologically distinguishable from the ancestral species (e.g. *Myzus persicae* and the permanently parthenogenetic and karyotypically re-arranged *Myzus antirrhinii*, Blackman & Paterson 1986). They argued further that continuously parthenogenetic entities diverging from each other by mutation should not be considered separate species. In the present case, microsatellite analysis has demonstrated the close genetic similarity between Australian *S. miscanthi* with derived karyotypes (Sunnucks *et al*. 1996; Wilson *et al*. 1999, Fig. 4): these are presumably derived from a recent common ancestor by mutation under apomixis and under Blackman and Brown (1991) criteria would represent one new species rather than several despite the demonstration of their divergent morphology described

Table 6 Proportion of contribution and variable coefficients of first three eigenvectors for principal component analysis of sexual forms of *Sitobion miscanthi*, *Sitobion fragariae*, *Sitobion* near *fragariae* and *Sitobion avenae*, and total sample standardised canonical coefficients for canonical discriminant analysis with four groups as classification values

	Principal component 1	Principal component 2	Principal component 3	Canonical axis 1	Canonical axis 2	Canonical axis 3
A – oviparae						
Proportion of total variation	52%	19%	9%			
htib	0.355	–0.099	0.037	1.04319	0.63027	–1.5652
cauda	0.243	–0.279	–0.383	0.69644	–0.3378	–0.6631
siph	0.239	0.386	–0.367	–4.0121	0.77594	–2.0319
siphret	0.178	–0.537	0.019	0.47026	–0.1866	–0.5078
A3	0.363	–0.090	–0.152	0.10571	0.15235	1.55653
A4	0.345	–0.178	0.023	0.20443	–0.2246	–0.1466
A5	0.365	0.151	0.137	0.38832	–0.3095	0.56698
pt	0.253	0.258	–0.446	–0.2155	–0.8765	0.36877
A6b	0.188	0.383	0.345	0.21705	–0.0993	0.41615
ursl	0.319	0.111	–0.034	–0.042	0.09078	0.28539
htars2	0.281	–0.337	0.364	1.61277	0.24957	–0.469
phetT	0.249	0.272	0.473	0.56813	2.34554	0.62086
B – males						
Proportion of total variation	47%	16%	11%			
htib	0.393	–0.164	–0.114	0.253	1.910	0.353
siph	0.320	–0.353	–0.200	6.681	–2.092	1.557
siphret	0.203	–0.177	0.505	–0.476	–0.095	0.394
A3	0.390	–0.070	–0.074	–0.376	0.933	–2.929
A3rh	0.218	0.369	–0.104	–0.836	0.277	0.400
A4	0.393	–0.002	–0.049	–1.097	0.542	–0.402
A4rh	0.095	–0.428	0.445	–0.157	–0.084	0.850
A5	0.387	0.046	0.014	–0.297	–0.584	0.424
A5rh	0.019	0.466	0.481	–0.367	–0.249	0.572
A6b	0.206	0.306	0.397	0.383	–1.290	–1.218
ursl	0.300	0.039	0.027	0.090	–0.460	–0.014
htars2	0.237	0.422	–0.292	–0.298	2.906	0.627

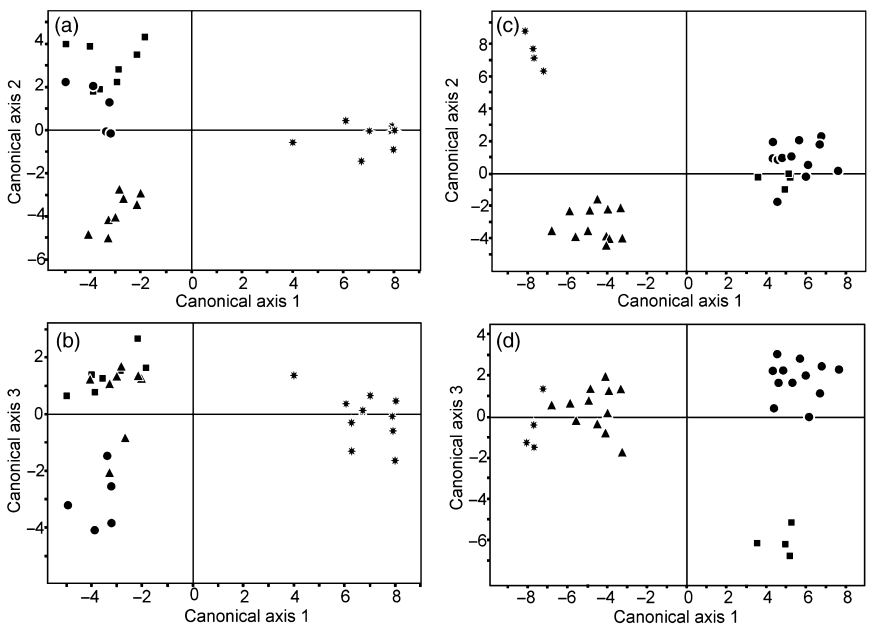

Fig. 6. Ordination of sexual forms on first three canonical discriminant analysis axes of four *Sitobion* classes. (a,b) oviparae; (c,d) males; *Sitobion miscanthi* (▲), *Sitobion avenae* (*), *Sitobion fragariae* (□), *Sitobion* near *fragariae* (●).

herein, different latitudinal distributions, temperature and host relations (Sunnucks *et al*. 1996, 1998; Turak *et al*. 1998). We have indicated elsewhere (Sunnucks *et al*. 1996) that *S. miscanthi* in Australia represents two separate successful introductions, one with the 2n = 18 karyotype and one with the re-arranged and rapidly evolving 2n = 17, 20, 21 group. Similar lineages are present in New Zealand, but New Zealand has an additional group of genotypes associated with re-arranged karyotypes. Members of the latter group are also closely related to each other but form a sister group to the Australian forms, having a separate Asian origin, and could therefore by Blackman and Brown's criteria be considered another separate species.

As with *S. fragariae*, most of the samples measured represent single lineages, and it might be considered desirable to validate the morphometric distinctions noted here with further karyotyped and genetically analysed material collected from the field, before undertaking species description. It should be noted, however, that of 555 independently collected Australian *S. miscanthi* genotyped at four microsatellite loci, all fell into only four genotype groups precisely correlated with karyotype where karyotype was also available, and variation within genotype groups was slight and rare, even after the addition of a further 10 microsatellite loci (Sunnucks *et al*. 1996; Wilson *et al*. 1999). Because of the small number of genotypes present in Australia and New Zealand, the range of morphometric variation within Australasia may be limited regardless of the number of samples measured, although the species is much more genotypically variable in Asia (Wilson *et al*. 1999; Wang *et al*. 2008).

A further complication within the *S. fragariae*/*S. avenae*/*S. miscanthi* group is the well-established introgression of genes between *S. fragariae* and *S. avenae* (Sunnucks *et al*. 1997) and perhaps also *S. fragariae* and *S. miscanthi* (see S?.NZ1 specimen in Wilson *et al*. 1999, and difficulties in identifying some New Zealand material; DAJ Teulon pers. comm. 1999). Naming yet more entities within the species group does not appear to be a useful activity, and we prefer to distinguish within *S. miscanthi* on the basis of karyotypic or molecular data rather than as named species.

Sexual morphs

The CDA was able to separate all four taxa in both sexes, but the results for oviparae may be misleading because of the likelihood of intermediate expression of oviparoid features in the laboratory-reared *S. miscanthi* and, to a smaller extent, *S.* nr *fragariae*, and thus do not support or contradict the placement of *S.* nr *fragariae* in *S. fragariae*. Intermediates between oviparae and parthenogenetic females have been observed on many occasions in field-collected aphids and can be produced in the laboratory by manipulation of environmental or physiological conditions (examples: Lees 1978; Searle & Mittler 1982; Corbitt & Hardie 1985; Hardie & Lees 1985; Takahashi & Inaizumi 1989). Parthenogenetic females may have scent plaques, reproductive systems may contain both eggs and embryos, and oviparae may even display the reproductive behaviour of parthenogenetic females. Males, however, express a less variable phenotype, as their determination is chromosomal rather than hormonal (Orlando 1974; Blackman & Hales 1986). The very clear separation of *S.* nr *fragariae* from *S. fragariae* males on CDA axis 3 may result from the bottleneck effect of the presumed single introduction of this taxon to Australia. Overall, the analysis of sexual morphs is less robust than that for parthenogenetic morphs because the number of available specimens and the number of samples was much smaller. The sexual specimens of *S. miscanthi* described above are not designated as morphotypes because of their laboratory origin. Voucher specimens are available in ANIC.

Synonymy of *S. miscanthi*, *S. akebiae* and *S. avenae*?

Choe *et al*. (2006) proposed that the species *S. miscanthi* and *S. akebiae* should be synonymised under *S. avenae*, on the basis of PCA and some molecular data. From their Figure 1, however, it appears that there is a degree of separation among the species, and the more appropriate CDA as used in the present paper might well separate them more fully. The mtCOII fragment used by Choe *et al*. is not very variable and therefore not especially useful in discriminating among closely related aphids: e.g. it fails to discriminate among the *Myzus persicae* group (CJ Metcalfe unpubl. data 1995; RL Blackman pers. comm. 1998). Besides this, all *Sitobion* have nuclear transpositions (pseudogenes) of mitochondrial DNA and these can give rise to misleading sequences (Sunnucks & Hales 1996). Choe *et al*. (2006) used only one sequence for *S. miscanthi* (that provided by Sunnucks & Hales 1996), and two sequences for *S. avenae* (one from England and one from North America). Ideally, a greater geographic spread of samples and more amenable DNA regions should be used: many are now available given the huge growth in published aphid genome sequence. Thus, we choose not to follow the suggestion of Choe *et al*. (2006) to subsume *S. miscanthi* in *S. avenae* at this time, pending a thorough revision of Asian *Sitobion*.

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