

Genetic structure and long-distance dispersal in populations of the wingless pest springtail, *Sminthurus viridis* (Collembola: Sminthuridae)

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(Received 7 July 2010 and in revised form 15 October 2010; first published online 11 January 2011)

Summary

The lucerne flea, *Sminthurus viridis* (Collembola: Sminthuridae) (L.) is a major pest of broadacre agriculture across southern Australia. Few molecular studies have been conducted on *S. viridis* and none have examined its population genetics, despite the importance for developing effective control strategies. Here, we characterize the genetic structure of Australian populations using three allozyme and eight microsatellite loci, as well as sequencing a fragment of the mitochondrial DNA cytochrome oxidase I gene. We found that *S. viridis* in Australia are diploid, sexually reproducing and exhibit significant population structure as a result of limited gene flow. Despite significant differentiation between populations, there was very low cytochrome oxidase subunit I (COI) gene sequence variation, indicating the presence of a single species in Australia. The observed structure only marginally complied with an ‘isolation by distance’ model with human-mediated long-distance dispersal likely occurring. Allozymes and microsatellites gave very similar F_{ST} estimates, although differences found for novel alternative estimates of differentiation suggest that the allozymes did not capture the full extent of the population structure. These results highlight that control strategies may need to vary for locally adapted *S. viridis* populations and strategies aimed at limiting the spread of any future pesticide resistance will need to manage the effects of human-mediated dispersal.

1. Introduction

Collembola are rarely considered as economically important pests of agriculture. However, in the southern hemisphere, particularly in Australia, *Sminthurus viridis* (L.) (Collembola: Sminthuridae) is a major agricultural pest of broadacre crops and pastures (Bishop *et al.*, 2001). In the southern regions of Australia, *S. viridis*, which is commonly named the lucerne flea, is particularly injurious during establishment of winter grain crops and again in early spring. Previous research has focused on studying their life cycle (Davidson, 1934; Wallace, 1967; Bishop *et al.*, 2001) and assessing their susceptibility to pesticides (Bishop *et al.*, 1998; Roberts *et al.*, 2009), but there have been no population genetic studies conducted on this species.

Analysis of population processes can provide valuable insight into the evolution and ecology of species and there are numerous molecular tools available for such investigations (Hoy, 2003). Molecular markers, such as microsatellites and allozymes, are routinely used for elucidating the status of species, establishing modes of reproduction and gaining insight into population structure and gene flow (Handler & Beeman, 2003). This information is particularly important for pest species as cryptic species can undermine control strategies through their differing pesticide susceptibility, life cycle and plant hosts (Umina & Hoffmann, 1999; Umina *et al.*, 2004). Knowledge of gene flow is valuable in managing the spread of pesticide resistance and determining appropriate scales for control options (Lushai & Loxdale, 2004; Endersby *et al.*, 2006).

S. viridis is a wingless springtail with adults developing to approximately 3 mm in length. Since its inadvertent introduction from Europe in the late

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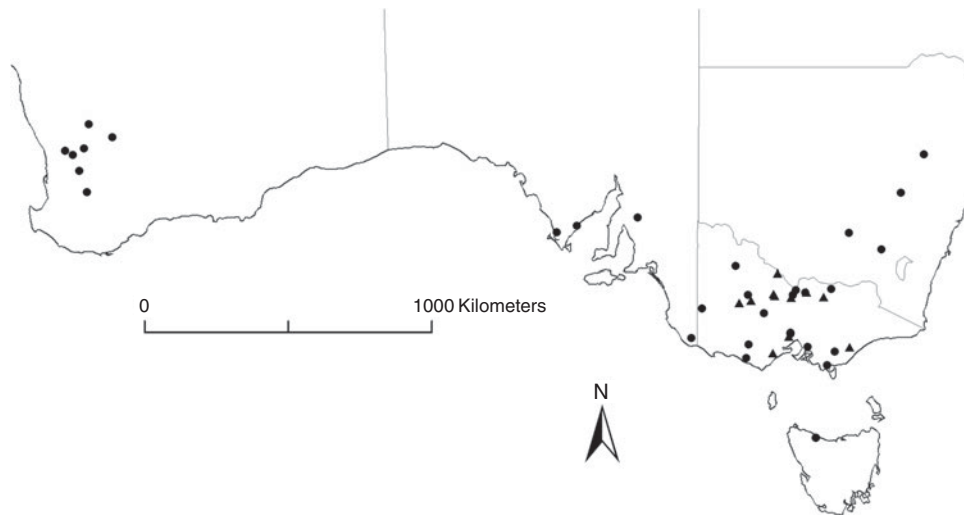


Fig. 1. Map of *S. viridis* sample populations across Australia used for allozyme (▲) and microsatellite (●) analysis. Australian states reading left to right are Western Australia, South Australia, New South Wales, Victoria and Tasmania.

1800s, it has become established across southern Australia in Mediterranean-type climates (Wallace & Mahon, 1971). This springtail has also invaded and become problematic in New Zealand, South Africa, South America and Asia (Wallace, 1974; Klein *et al.*, 2005). In Australia, *S. viridis* is a sporadic, winter-active pest that emerges in autumn from over-summering eggs after suitable rainfall and will undergo between two and five generations prior to the onset of summer (Bishop *et al.*, 2001). Observational evidence of sexual mating behaviour (Betsch-Pinot, 1976) and sex-ratio estimates (Davidson, 1934) indicate *S. viridis* is likely to reproduce sexually. Batches of around 60 eggs are laid on the surface of heavier loam soil types and are covered with a protective layer of glandular secretions by the female (Wallace, 1968). The main hosts of *S. viridis* are broad-leaved plants such as lucerne (*Medicago sativa*), clovers (*Trifolium* spp.) and capeweed (*Arctotheca calendula*); however, damage to seedlings of various grain crops is also common (Wallace, 1967).

Control options for *S. viridis* rely heavily on the prophylactic application of organophosphate pesticides (Roberts *et al.*, 2009, No. 198). The nature of these spraying practices is likely to cause genetic bottlenecks and create strong selection pressure for pesticide resistance alleles (Hoffmann *et al.*, 2008, No. 152). To date, there is no evidence of pesticide resistance in *S. viridis* populations, although they are known to have high chemical tolerances to several pesticides used for their control when compared with other establishment pests (Roberts *et al.*, 2009). Knowledge of the population structure and migration potential of *S. viridis* will be essential for managing pesticide resistance if it arises.

Here we have used allozymes, microsatellites and the mitochondrial DNA cytochrome oxidase subunit

I (COI) gene to examine the species status, mode of reproduction and population genetics of *S. viridis* in Australia. These marker systems have been routinely used for examining the population structure and gene flow of invertebrate pests for improving control strategies (Handler & Beeman, 2003). We contrast the patterns found with the different markers systems, particularly allozymes and microsatellites, and discuss the findings in the context of developing effective control strategies for *S. viridis*.

2. Methods

(i) Field sampling

Samples of *S. viridis* were collected throughout its Australian distribution between 2005 and 2007 (Fig. 1, Appendix 1 in Supplementary material). A total of 13 Victorian populations were sampled in 2005 for analysis with allozyme markers and 32 populations sampled in 2006 and 2007 across southern Australia were analysed with microsatellite markers. Samples were collected predominantly from pasture containing clover (*Trifolium* spp.), although samples were also collected from grain crops (e.g. oats), lucerne (*M. sativa*) and roadside weeds (mainly capeweed, *A. calendula* and barley grass, *Hordeum leporinum*). Collections were made by suction sampling with a Stihl SH55 blower vacuum and taken to the laboratory in plastic containers with plant material and paper towel. Adults were then isolated from the sample and stored at -70°C .

(ii) Allozymes

We screened 38 allozyme loci for variability in *S. viridis* using the Titan III cellulose acetate gel electrophoresis

system of Helena Laboratories (Hebert & Beaton, 1989). Of these, seven were monomorphic, whereas three loci displayed polymorphic and interpretable variability; mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 5.4.2.2) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44). All other loci either did not stain or displayed uninterpretable banding patterns. Briefly, an individual *S. viridis* adult was placed with 6 μ l of sterile distilled water into each well of the sample plate and crushed with a spatula. Cellulose acetate gels (76 mm \times 76 mm) were soaked in running buffer for 20 min, blotted dry on filter paper and then placed on the aligning base. Extracts were simultaneously applied either centrally (cathodally migrating enzymes) or basally (anodally migrating enzymes) to the gel from each sample well using an applicator (Helena Laboratories). Gels were then positioned in electrophoresis tanks with the extracts at the anode end (where applicable) and the gels surface touching paper wicks at each end. Gels were run at 220 V for 20 min using a Bio-Rad Power/Pac 3000 power supply. Following this, gels were placed on glass plates and a stain consisting of 2 ml of stain ingredients and 6 ml of 1% agarose was applied to the gel surface. All stain recipes were taken from Hebert & Beaton (1989) or Richardson *et al.* (1986) with some modifications. Following staining of the gel for a specific enzyme, the resulting banding pattern was recorded.

(iii) *Microsatellites*

DNA was extracted from whole *S. viridis* adults using a CTAB (hexadecyltrimethylammonium bromide) protocol involving chloroform/isoamylalcohol (24:1), proteinase K and ethanol precipitation with 5 M NaCl (Roberts & Weeks, 2009). Samples were genotyped using eight microsatellite loci described in Roberts & Weeks (2009) and amplified with the same PCR mixtures and cycling conditions (with [γ^{33} P]-ATP end labelled forward primer). PCR products were then separated through 5% polyacrylamide gels at 65 W for \sim 2 h and exposed for 24 h to autoradiographic film (OGX, CEA, Strängnäs, Sweden).

(iv) *Mitochondrial DNA*

A 437 bp fragment of the mitochondrial COI gene was amplified using *S. viridis*-specific primers (5'-CCTGGAAGATTAATTGGAGACG-3'/5'-TCAA-AACAAGTGTGGTATAGAATAGG-3'). These primers were designed to be specific for the COI mitochondrial DNA gene of *S. viridis* in Australia by using sequence data from several individuals obtained with universal invertebrate primers described by Folmer *et al.* (1994). DNA extracted with a CTAB protocol was amplified in a 30 μ l reaction containing

1 \times PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M forward primer, 0.2 μ M reverse primer, 0.6 units *Taq* polymerase (New England Biolabs) and 4 μ l template DNA. PCR cycling conditions were 35 cycles of 94 $^{\circ}$ C (30 s), primer annealing at 50 $^{\circ}$ C (1 min) and extension at 72 $^{\circ}$ C (1 min) using an Eppendorf Mastercycler 534 \times . The COI PCR products were sequenced by Macrogen Inc. (Seoul, South Korea) in both directions and sequence data aligned, edited and translated in MEGA version 4.0 (Tamura *et al.*, 2007). All unique sequences have been deposited in GenBank (Accession numbers: HM355586–HM355589).

(v) *Data analysis for allozymes and microsatellites*

Genetic Data Analysis version 1.0 (Lewis & Zaykin, 2001) was used for estimating observed (H_O) and expected (H_E) heterozygosity, Weir & Cockerham's (1984) inbreeding coefficient (F_{IS}) and a global estimate of population differentiation (F_{ST}), as well as testing for deviations from Hardy–Weinberg equilibrium within populations and loci. Allelic richness averaged over loci, population pairwise F_{ST} estimates and linkage disequilibrium between loci was determined with FSTAT version 2.9.3 (Goudet, 2001). Additional estimates of population differentiation, G'_{ST_est} , G'_{ST_est} and D_{est} were obtained using SMOGD: software for the measurement of genetic diversity (Crawford, 2010). These estimates are suggested to be more accurate for identifying population structure by overcoming the limitations of F_{ST} (Jost, 2008). POWSIM version 4.0 (Ryman & Palm, 2006) was used for evaluation of the alpha error and statistical power of the allozyme and microsatellite loci for accurately detecting different levels of F_{ST} . Population differentiation was also investigated using hierarchical analyses of molecular variance (AMOVA) in Arlequin version 3.11 (Excoffier *et al.*, 2005), using pairwise F_{ST} as the distance measure, with 10 000 permutations and missing data for loci set at 10%. The model used for the full microsatellite data partitioned the total variance among regions (Victoria, Western Australia, South Australia, New South Wales and Tasmania), among populations within regions and within populations. For the temporal data the model used partitioned the total variance among populations, among samples within populations and within samples. The model used for the Victorian microsatellite data and allozyme data partitioned the total variance among populations and within populations.

Bayesian clustering methods were used in Structure version 2.3.1 (Pritchard *et al.*, 2000) and Structurama (Huelsenbeck & Andolfatto, 2007) for identifying population structure and estimate the number of populations in the microsatellite data. In Structure version 2.3.1, a burn-in length of 10 000 followed by

10 000 iterations was used for each simulation using an admixture model and correlated allele frequencies among populations. The method of Evanno *et al.* (2005) was used for estimating the true number of populations in the data, K' . In Structurama, 10 000 Markov chain Monte Carlo cycles were used for inferring K following a Dirichlet process. This model assumes that the number of populations and the expected prior number of populations were random variables with a gamma distribution of shape and scale equal to 1 (Huelsenbeck & Andolfatto, 2007). Patterns of genetic variation were also visualized by a factorial correspondence analysis performed in Genetix version 4.05.2 (Belkhir *et al.*, 2004) with the first two factors explaining the majority of the variation in multilocus genotypes plotted. Gene flow between populations was estimated using the F_{ST} value averaged over all loci and the formula, $Nm = (1 - F_{ST})/4F_{ST}$ (Slatkin & Barton, 1989), where N is the effective population size and m is the migration rate. Linearized F_{ST} and Nei's genetic distance D were also plotted against natural log geographic distance and tested for compliance with an 'isolation by distance' model by regression and Mantel tests. To test for signals of possible recent population bottleneck or founder events in the microsatellite data, the program Bottleneck version 1.2.02 was used (Piry *et al.*, 1999). The program tests for a significant heterozygote excess compared to the heterozygosity level expected from the population sample size and allelic diversity. Dramatic reductions in effective population size are predicted to reduce allelic richness, through loss of rare alleles, more quickly than heterozygosity. One-tailed Wilcoxon tests were used under a two-phase model with 95% single-step mutations, 5% multi-step mutations and a variance among multiple steps of 12. Where needed, sequential Bonferroni corrections for multiple comparisons at the table-wide $\alpha = 0.05$ level were undertaken.

3. Results

(i) Allozyme analysis

From the 13 Victorian populations analysed, a total of seven alleles were found for the three polymorphic allozyme loci (Table 1) with an allelic richness of 2.33 in all populations except for Sale ($r = 2.00$) (Table 2). This population also had the lowest observed and expected heterozygosity (0.351 and 0.307, respectively). All populations, however, showed moderate levels of observed and expected heterozygosity, typical of allozyme markers (H_O , 0.351–0.493; H_E , 0.307–0.519). No population had F_{IS} values that were significantly different from zero or displayed significant departures from Hardy–Weinberg equilibrium (Table 2). There was also no linkage disequilibrium

Table 1. Molecular markers used to screen Australian populations of *S. viridis*. Overall values of populations for sample size (n), allelic number (a), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and Hardy–Weinberg equilibrium P -values are given

Locus	n	a	H_O	H_E	F_{IS}	HWE- P
<i>Allozymes</i>						
PGM	764	3	0.418	0.466	0.103	0.566
MPI	762	2	0.398	0.460	0.137	0.491
6PGDH	745	2	0.506	0.485	−0.043	0.622
<i>Microsatellites</i>						
SV05	950	7	0.734	0.780	0.059	0.543
SV10	953	3	0.454	0.494	0.080	0.717
SV19	954	7	0.647	0.739	0.125	0.497
SV20	964	2	0.415	0.451	0.080	0.620
SV24	981	4	0.563	0.602	0.065	0.477
SV27	989	6	0.597	0.684	0.127	0.440
SV30	998	4	0.635	0.676	0.061	0.460
SV34	989	5	0.537	0.590	0.091	0.476

observed between loci across populations with all 39 pairwise tests being non-significant after correction for multiple comparisons.

The estimate for F_{ST} over all loci was 0.063 (0.022–0.111, 95% CI), which suggests significant population structure and limited gene flow exists between these samples. Similarly, significant population structure was shown by the estimates of G'_{ST_est} , G'_{ST_est} and D_{est} which ranged from 0.056 to 0.114 (Table 3). Significant variation among these samples was also shown in an AMOVA with 6.3% ($P < 0.001$) of the variation explained by variation among populations and 93.7% of the variation explained by variation within populations (Table 4). Population pairwise comparisons of F_{ST} revealed only moderate differentiation between these Victorian samples with 35 of 78 comparisons being non-significant after correction for multiple comparisons (Appendix 2 in Supplementary material). However, two populations (Echuca and Sale) showed complete differentiation from all populations and the Melton population was differentiated from all but one population. This pattern of genetic variation was supported by a factorial correspondence analysis where the first two axes explained 60.9 and 16.8% of the variation (Fig. 2(a)). Three populations (Echuca, Sale and Melton) are clearly differentiated from the other populations. However, no significant correlation was found between Slatkin's linearized F_{ST} (Mantel $r = 0.195$, $P = 0.136$; $R^2 = 0.004$, $F = 0.297$, $P = 0.587$) or Nei's genetic distance D (Mantel $r = 0.195$, $P = 0.136$; $R^2 = 0.004$, $F = 0.297$, $P = 0.587$) against the natural log of geographic distance. Gene flow estimates using the formula, $Nm = (1 - F_{ST})/4F_{ST}$, indicate a migration

Table 2. Population statistics for *S. viridis* analysed with allozyme and microsatellite markers. Overall loci values for sample size (n), allelic richness (r), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and Hardy–Weinberg equilibrium P -values are given

Population	n	r	H_O	H_E	F_{IS}	HWE- P
<i>Allozymes</i>						
Barpinba, VIC	66	2.33	0.424	0.399	-0.063	0.471
Bears Lagoon 2, VIC	66	2.33	0.374	0.398	0.062	0.750
Benalla, VIC	66	2.33	0.482	0.451	-0.069	0.341
Deans Marsh, VIC	24	2.33	0.431	0.434	0.008	0.624
Echuca, VIC	66	2.33	0.482	0.519	0.071	0.729
Elmore, VIC	66	2.33	0.393	0.455	0.137	0.576
Koondrook, VIC	66	2.33	0.433	0.461	0.060	0.698
Melton, VIC	31	2.33	0.532	0.528	-0.008	0.704
Rupanyup, VIC	66	2.33	0.465	0.490	0.052	0.609
Sale, VIC	66	2.00	0.351	0.307	-0.145	0.502
St. Arnaud, VIC	66	2.33	0.439	0.469	0.064	0.438
Serpentine, VIC	66	2.33	0.493	0.447	-0.104	0.282
Serpentine 2, VIC	60	2.33	0.450	0.425	-0.058	0.553
<i>Microsatellites</i>						
Bringalbert, VIC	31	3.68	0.620	0.604	-0.026	0.534
Corop, VIC	31	3.60	0.608	0.605	-0.005	0.397
Cranbourne, VIC	31	3.34	0.505	0.494	-0.023	0.700
Darlington, VIC	29	3.57	0.567	0.558	-0.017	0.763
Donald, VIC	31	3.54	0.607	0.597	-0.016	0.542
Echuca 2, VIC	32	3.39	0.513	0.551	0.071	0.472
Fish Creek, VIC	27	3.47	0.623	0.626	0.004	0.595
Lascelles, VIC	31	3.66	0.633	0.633	-0.001	0.446
Melton 2, VIC	31	3.31	0.540	0.554	0.025	0.628
Peechelba, VIC	31	3.56	0.598	0.605	0.012	0.469
Timboon, VIC	29	3.36	0.557	0.578	0.036	0.317
Toolern Vale, VIC	32	3.44	0.641	0.617	-0.039	0.460
Traralgon, VIC	31	3.37	0.525	0.556	0.058	0.317
Undera, VIC	31	3.45	0.600	0.569	-0.054	0.729
Wareek, VIC	32	3.44	0.566	0.571	0.008	0.579
Brookton, WA	27	3.44	0.563	0.560	-0.006	0.460
Clackline, WA	31	3.46	0.557	0.574	0.030	0.640
Cunderin, WA	30	3.49	0.574	0.580	0.010	0.460
Highbury, WA	32	3.67	0.573	0.596	0.040	0.391
Koorda, WA	32	3.03	0.508	0.490	-0.038	0.751
Merredin, WA	30	3.43	0.584	0.564	-0.037	0.565
York, WA	31	3.73	0.589	0.615	0.041	0.361
Ardlethan, NSW	30	3.34	0.556	0.570	0.025	0.410
Coolac, NSW	30	2.75	0.507	0.498	-0.018	0.501
Coolah, NSW	31	3.72	0.648	0.632	-0.025	0.585
Molong, NSW	30	3.12	0.513	0.514	0.003	0.478
Clare, SA	30	3.60	0.607	0.603	-0.007	0.656
Coulta, SA	32	3.35	0.523	0.518	-0.010	0.361
Mt. Gambier, SA	31	3.58	0.573	0.576	0.006	0.461
Tumby Bay, SA	32	3.74	0.629	0.633	0.006	0.582
Elliot, TAS	29	3.46	0.546	0.583	0.063	0.488
Elliot 2, TAS	27	3.24	0.564	0.561	-0.007	0.571

rate of 3.72 individuals per generation between these populations.

(ii) *Microsatellite analysis*

A total of 38 alleles were found for the eight microsatellite loci in samples from 32 Australian locations (Table 1). Average allelic richness ranged from 2.75 to 3.74, although all but one population had an allelic

richness greater than 3 (Table 2). Heterozygosity was moderately high in all populations with estimates ranging from 0.505 to 0.648 and 0.490 to 0.633 for observed and expected heterozygosity, respectively. For all populations F_{IS} was not significantly different from zero and there were no significant departures from Hardy–Weinberg equilibrium (Table 2). No consistent linkage disequilibrium was found between loci across populations, with only three significant

Table 3. Estimates of differentiation between *S. viridis* populations and their 95% confidence intervals obtained from three allozymes and eight microsatellite markers

	Allozymes		Microsatellites VIC		Microsatellites All	
		95% CI		95% CI		95% CI
F_{ST}	0.063	0.022–0.111	0.067	0.051–0.083	0.086	0.063–0.109
G_{ST_est}	0.063	0.051–0.093	0.062	0.055–0.101	0.096	0.081–0.113
G'_{ST_est}	0.114	0.094–0.167	0.165	0.149–0.247	0.237	0.206–0.270
D_{est}	0.056	0.045–0.083	0.110	0.100–0.163	0.158	0.138–0.179

Table 4. Analysis of the molecular variation at three allozyme and eight microsatellite markers among regions (Western Australia, South Australia, Victoria, New South Wales and Tasmania), among populations within regions and within populations of *S. viridis*

Source of variation	d.f.	Sum of squares	Variance components	% Variation	Fixation indices	<i>P</i> -value
<i>Allozymes</i>						
Among populations	12	70.444	0.045	6.298	0.063 (FST)	<0.001
Within populations	1537	996.813	0.665	93.702	–	–
Total	1549	1067.256	0.709	–	–	–
<i>Microsatellites – Vic populations</i>						
Among populations	14	176.135	0.167	6.708	0.067 (FST)	<0.001
Within populations	927	2098.440	2.324	93.292	–	–
Total	941	2274.575	2.419	–	–	–
<i>Microsatellites – all populations</i>						
Among regions	4	104.048	0.036	1.430	0.014 (FCT)	<0.001
Among populations within regions	27	376.024	0.191	7.550	0.077 (FSC)	<0.001
Within populations	1970	4393.922	2.298	91.020	0.090 (FST)	<0.001
Total	2001	4873.994	2.525	–	–	–
<i>Temporal samples – microsatellites</i>						
Among populations	1	59.204	0.301	11.583	0.116 (FCT)	<0.001
Among samples within populations	6	11.586	–0.007	–0.281	–0.003 (FSC)	0.844
Within samples	376	859.021	2.304	88.699	0.094 (FST)	<0.001
Total	383	929.811	2.597	–	–	–

tests out of 896 pairwise comparisons after correction for multiple comparisons. Evidence of a recent population bottleneck was not found in any population, with no significant heterozygote excess detected after correcting for multiple comparisons.

Estimates of population differentiation for all the Australian samples indicated significant structure and limited gene flow between populations. The estimate for F_{ST} over all loci was 0.086 (0.063–0.109, 95% CI), whereas estimates for G_{ST_est} , G'_{ST_est} and D_{est} were somewhat higher ranging from 0.096 to 0.237 (Table 3). Estimates of population structure for only the Victorian populations showed similar results to the allozyme data with an F_{ST} over all loci of 0.067 (0.051–0.083, 95% CI) and estimates for G_{ST_est} , G'_{ST_est} and D_{est} ranging from 0.062 to 0.165 (Table 3). Significant genetic variation between the Australian samples was also shown from an AMOVA analysis with the majority of variation explained by variation within populations (91%, $P < 0.001$), 7.6% ($P < 0.001$) of the variation explained by variation among

samples within regions and 1.4% ($P < 0.001$) of the variation explained by variation among regions (Table 4). An AMOVA for only the Victorian populations showed 6.7% ($P < 0.001$) of the variation was explained by variation among samples and 93.3% ($P < 0.001$) of the variation was explained by variation within samples (Table 4). Maintenance of this genetic variation over time was also shown from an AMOVA analysis, with no significant variation found among four temporal samples taken from two different populations over a two-year period ($P = 0.884$). Substantial differentiation was found for the population pairwise F_{ST} estimates with only 21 out of 496 tests being non-significant after correction for multiple comparisons (Appendix 3 in Supplementary material). For the Victorian population pairwise F_{ST} estimates only 2 out of 105 tests were non-significant after correction for multiple comparisons (Appendix 3 in Supplementary material).

The pattern of genetic variation for these Australian samples was visualized by plotting the first two factors

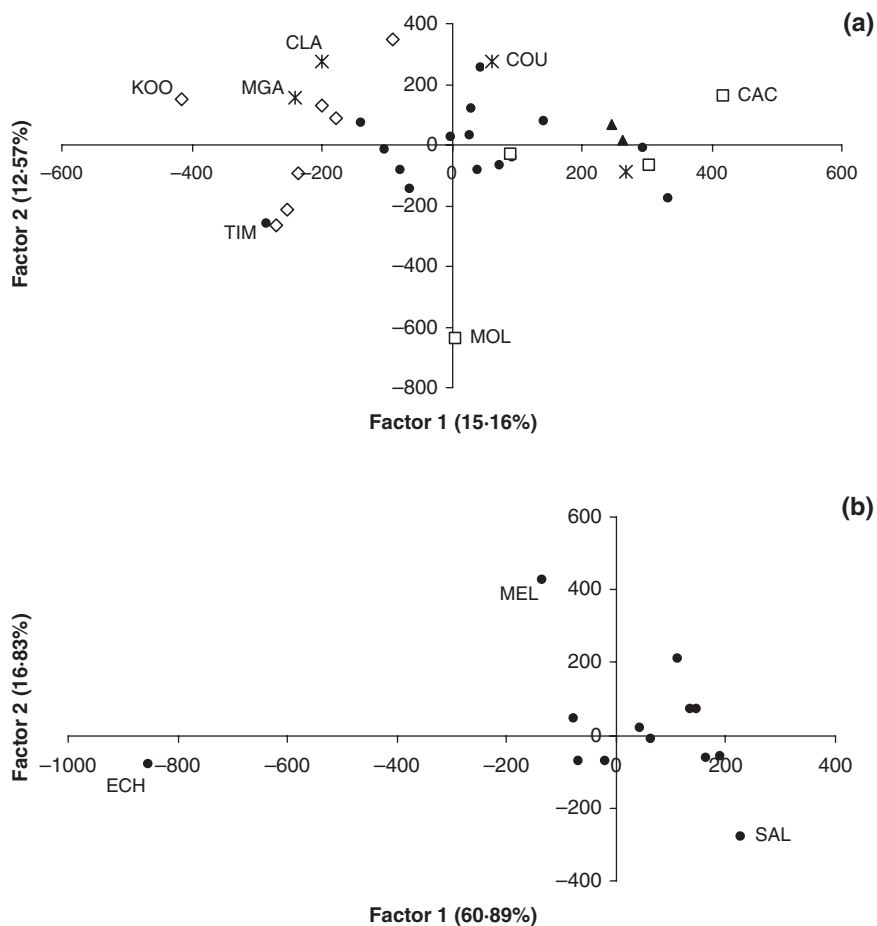


Fig. 2. Factorial correspondence analysis by populations for *S. viridis*. Each point represents a sample region weighted by the number of individuals and the sum of alleles present. (a) Allozyme data; percentage of variation explained by the first factor is 60.89%. ECH = Echuca, VIC; MEL = Melton, VIC; SAL = Sale, VIC. (b) Microsatellite data; open diamonds = Western Australian population samples, star = South Australian population samples, closed circles = Victorian population samples, open squares = New South Wales population samples, closed triangles = Tasmanian population samples. Percentage of variation explained by the first factor is 15.16%. CAC = Coolac, NSW; CLA = Clare, SA; COU = Coultia, SA; KOO = Koorda, WA; MGA = Mt Gambier, SA; MOL = Molong, NSW; TIM = Timboon, VIC.

(15.16 and 12.57%, respectively) explaining the majority of the variation in a factorial correspondence analysis (Fig. 2(b)). Appreciable genetic variation is apparent both between and within regions, although the Victorian samples seem more discrete. Also, there is still an overlap between regions, despite considerable geographic distance between some regions (e.g. Western Australia and Victoria). In particular, Timboon (VIC), Mount Gambier (SA) and Clare (SA) were closely associated with Western Australian populations. The results from the Structure analysis, using the Evanno *et al.* (2005) method for estimating the true K , and the Structurama analysis both indicate that there are four groups within the data set. However, this grouping appears largely due to a couple of very distinct populations. Coolac (NSW), Molong (NSW), Koorda (WA) and Coultia (SA) are each the main population contributing to each group. Several other populations show a moderate affinity

to these groups, although many show a strong admixture.

A significant correlation was found between Nei's genetic distance D and natural log geographic distance (Mantel $r=0.166$, $P=0.026$; $R^2=0.028$, $F=14.047$, $P<0.001$), but only marginal significance between Slatkin's linearized F_{ST} and natural log geographic distance (Mantel $r=0.140$, $P=0.075$; $R^2=0.020$, $F=9.867$, $P=0.002$) (Fig. 3(a)). The results suggest that Australian populations may comply somewhat with an 'isolation by distance' model, but there are other factors also dictating structure. A similar trend was shown for the Victorian populations although again only marginal significance was found for both Slatkin's linearized F_{ST} (Mantel $r=0.191$, $P=0.117$; $R^2=0.036$, $F=3.897$, $P=0.051$) (Fig. 3(b)) and Nei's genetic distance D (Mantel $r=0.197$, $P=0.091$; $R^2=0.039$, $F=4.160$, $P=0.044$). Gene flow estimates indicate a migration rate of 3.48 and 2.66

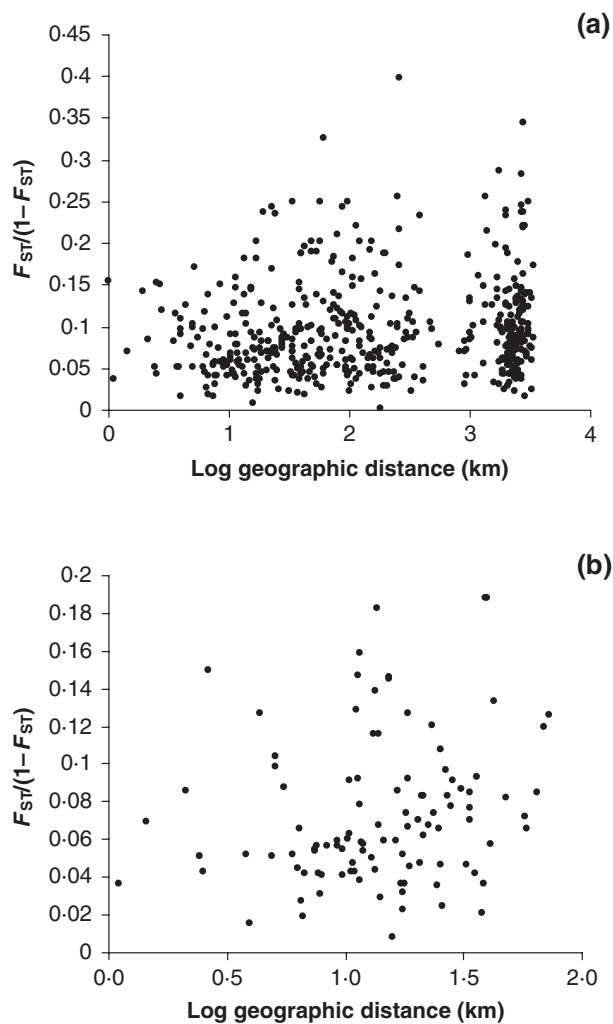


Fig. 3. Regression of linearized F_{ST} ($F_{ST}/1-F_{ST}$) and natural log geographic distance (km) for (a) all microsatellite-analysed Australian populations (Mantel $r=0.140$, $P=0.075$; $R^2=0.020$, $F=9.867$, $P=0.002$); and (b) all microsatellite-analysed Victorian populations (Mantel $r=0.191$, $P=0.117$; $R^2=0.036$, $F=3.897$, $P=0.051$).

individuals per generation between the Victorian and Australian populations, respectively.

(iii) Statistical power

The statistical power of the allozyme and microsatellite markers to detect various levels of true F_{ST} values between populations is shown in Table 5. This takes into account the sample sizes, number of loci and average allele frequencies of the data set. The results show that both marker sets will detect a true F_{ST} of 0.01 or larger with a probability of 99% or more. The microsatellite markers did show greater statistical power with a 95% probability of detecting a true F_{ST} as low as 0.005, whereas allozymes had only ~70% probability of detection. The alpha error (i.e. the probability of obtaining false significances when the

Table 5. Statistical power of the allozyme and microsatellite markers for detecting various true F_{ST} values with Fisher's exact test and chi-square tests when using the study loci, allele frequencies and sample sizes. The power is expressed as the proportion of simulations that are statistically significant at the 0.05 level

True F_{ST}	Allozymes		Microsatellites	
	Chi-square	Fisher	Chi-square	Fisher
0.0000	0.060	0.050	0.060	0.085
0.0002	0.070	0.075	0.060	0.090
0.0020	0.315	0.290	0.605	0.550
0.0050	0.725	0.700	0.995	0.995
0.0075	0.930	0.920	1.000	1.000
0.0100	0.990	0.990	1.000	1.000

true $F_{ST}=0$) was also close to 5% for both marker sets.

(iv) Mitochondrial DNA analysis

A total of 65 *S. viridis* individuals from across its Australian distribution were sequenced for a 437 bp fragment of the mitochondrial *cytochrome oxidase I* gene. Extremely low sequence variation was found between samples with 89% of individuals belonging to a single haplotype (A). Three other haplotypes (B–D) were found although each differed from haplotype A by only a single bp substitution. All individuals from Tumbly Bay, SA were haplotype B, whereas haplotype C and D were found in single individuals from Mount Gambier, SA and Donald, VIC, respectively, alongside haplotype A. Comparison between haplotype A and sequence data of *S. viridis* COI gene obtained from GenBank (Accession number: EU016192) revealed a 17% sequence divergence between the two sequences.

4. Discussion

The presented data indicate that *S. viridis* is a single, diploid species that reproduces sexually throughout its Australian distribution. Allele frequencies within populations conformed to Hardy–Weinberg proportions and remained so between temporal samples, as is expected for sexually reproducing organisms. These results support earlier evidence of sexual mating where indirect sperm transfer was observed in laboratory-reared populations of European *S. viridis* (Betsch-Pinot, 1976) and also from sex-ratio estimates reported by Davidson (1934) in South Australia. Sexual reproduction is common within the Collembola group and is the only form of reproduction to be

found in the family Sminthuridae. There were also no fixed allelic differences between populations at any loci or significant variation at the mtDNA level, suggesting that all samples in this study represent a single species. However, there was substantial sequence divergence between *S. viridis* in Australia and the European sequence submitted to GenBank. The level of divergence is equivalent to that seen between Collembola genera. As we are confident that their taxonomic identification is accurate, it is unclear why there is such disparity between the sequences, but it is possible that they may represent cryptic species.

The data indicate that there is limited gene flow between populations of *S. viridis* in Australia, resulting in significant population structure. However, only a weak relationship was observed between genetic distance (linearized F_{ST} and Nei's D) and geographic distance in the Victorian and in the Australia wide samples. This relationship seems to be largely disrupted by instances where distant populations are less differentiated than those more proximate. Being a relatively recently introduced species (~100 years ago), populations may not be at mutation-drift equilibrium and therefore identity by descent may account for some genetic similarity, particularly between distant populations (Hartl & Clark, 2007). However, there may also be long-distance dispersal events occurring to some degree, which obviates normal adult dispersal.

The most likely mechanism providing long-distance dispersal is human-facilitated movement of *S. viridis* eggs and live stages with the transportation of animal fodder, seeds and soil (Davidson, 1934). It is through these means that *S. viridis* was introduced to Australia and other countries (Davidson, 1934) and is likely to continue to be an important means of dispersal. Long-distance gene flow could also occur through wind dispersal of diapause eggs (Alvarez *et al.*, 1999). These eggs are highly resistant to desiccation, withstanding months of dry conditions before suitable moisture is available (Wallace, 1968). However, if this were the primary mechanism for dispersal there would be greater gene flow expected between more proximate populations and an expectation that gene flow follows an isolation by distance model.

The factorial correspondence analysis and Structure analysis both showed the Western Australian populations to be differentiated from the majority of eastern Australian populations. This is not unexpected considering the large geographical barrier of the unsuitably dry environment separating Western Australia from the eastern populations. Interestingly, Timboon from south-western Victoria and also Clare and Mount Gambier from South Australia are closely associated with the Western Australian populations. Historically, *S. viridis* was first recorded in South Australia and subsequently introduced to Western

Australia with transported clover seed (Davidson, 1934). Furthermore, the first occurrences of *S. viridis* in Victoria were confined to the south-western region (Pescott, 1937). Although these patterns of variation may reflect historical gene flow, the genetic similarity of these populations suggests that more recent human-mediated dispersal is also occurring. The lack of variation between populations found at the mitochondrial COI gene further supports the notion that a small *S. viridis* population was introduced to Australia and has since radiated to its current distribution.

Several populations were also found to be strongly differentiated from the majority of populations and displayed some of the lowest levels of allelic richness and heterozygosity. Interestingly, those populations with low genetic diversity all occur near the distribution limits for *S. viridis* (Wallace & Mahon, 1971). This pattern coincides with ecological theory (Sexton *et al.*, 2009) and numerous empirical studies (Robinson *et al.*, 2002; Eckert *et al.*, 2008) that have found reduced genetic diversity and increased genetic differentiation of peripheral populations compared to central populations. Suboptimal and extreme conditions at distribution limits are likely to reduce effective population sizes and increase the occurrence of bottlenecks and founder events, which exacerbate the effects of genetic drift. Similarly, the sporadic nature of *S. viridis* and use of pesticides are also likely to result in bottlenecks throughout its range and contribute to the overall genetic structuring of populations. However, the Bottleneck analysis did not indicate whether any population had recently experienced a bottleneck or founder event. The signature of these events deteriorates after only two to four generations and may be difficult to detect in *S. viridis*, which has at least two generations a year (Bishop *et al.*, 2001).

Similar findings of genetic structure have been found between populations of several other species of Collembola. Studies by Fanciulli *et al.* (2001) and Stevens & Hogg (2003) have both found significant structure of populations in two different Antarctic species. Unlike *S. viridis*, glaciers have been important geographical barriers to gene flow in these cases. However, substantial population differentiation over shorter distances was also reported. Stevens & Hogg (2003) propose that human- or bird-mediated dispersal may account for the limited gene flow that is occurring. The remaining studies of population genetics for Collembola species have been conducted in Europe. Several studies conducted on *Orchesella cincta* have all found significant but often low differentiation of populations at various geographical scales (Timmermans *et al.*, 2005; Van Der Wurff *et al.*, 2005). In explanation of why stronger population structure was not observed for this wingless and

sedentary organism, the authors propose wind dispersal of individuals as facilitating long-distance gene flow. Evidence of stronger population differentiation was observed by Fanciulli *et al.* (2000) in four Collembola species from the genera *Tomocerus* and *Pogonognathellus*, with no evidence of long-distance dispersal occurring. Although it seems intuitive that genetic differentiation is somewhat characteristic of Collembola populations, passive long-distance dispersal may also be a more common feature of this group than previously thought.

Genetic structure has also been assessed for the sympatric mite pest, *Halotydeous destructor* (Weeks *et al.*, 1995). This introduced species has a similar life cycle and distribution to *S. viridis*, but is more ubiquitous in its distribution and has a broader host-plant range. Based on allozyme variation, Weeks *et al.* (1995) found lower levels of differentiation between *H. destructor* populations ($F_{ST}=0.015$) than that found here for *S. viridis*. Still, substantial heterogeneity of populations discounted the presence of a single panmictic unit. Similar to *S. viridis*, gene flow between *H. destructor* populations is likely greatly facilitated through human-mediated dispersal. However, due to a greater area of suitable host-plants throughout their range, *H. destructor* is likely to have larger effective population sizes that will reduce the effects of genetic drift.

Insight into the population genetics of *S. viridis* has important implications for the development of effective control strategies and pesticide resistance management. The observed population structure has the potential to undermine control strategies that are generalized over large areas. The *S. viridis* life cycle is an important component in targeted control strategies, but there may be significant phenotypic plasticity present throughout its distribution. Adaptive shifts in life-cycle characteristics could arise in differentiated populations in response to these control strategies. Similarly, current spraying practices are strongly selecting for pesticide resistance. Although there is currently no evidence of pesticide resistance in *S. viridis*, these results suggest that long-distance dispersal events could greatly increase the spread of resistance alleles. Therefore, it is likely that the effectiveness and sustainability of targeted control strategies will be greater when developed at smaller localized scales and future pesticide resistance management may need to incorporate measures for limiting human-mediated dispersal.

Similar to previous studies (Larsson *et al.*, 2007; Conte *et al.*, 2008) we found high concordance in F_{ST} estimates between allozymes and microsatellites. Allendorf & Seeb (2000) suggested the majority of differences between these marker systems can be attributed to a small number of outlier loci or alleles. While the statistical power of the microsatellites was

found to be greater than the allozymes, this difference was negligible at F_{ST} values estimated in this study. However, Jost (2008) argues that conventional measures of differentiation are inherently flawed and D_{est} provides a true measure of population structure. Estimates of Hedrick's G'_{ST} and Jost's D_{est} for the Victorian populations were substantially higher for the microsatellites than the allozymes. Therefore, it appears the allozyme markers were ineffective at capturing the full extent of the population structure. However, this result may be more a reflection of the number of loci used rather than their variability. Nevertheless, the differences between the allozymes and microsatellites and F_{ST} versus D_{est} are arguably of minor importance for this study as the overall pattern of differentiation remains the same.

This study has shown through the use of molecular markers that *S. viridis* in Australia reproduces sexually and has significant population structure as a result of limited gene flow. It also highlights the need for locally derived control strategies and the role that human-mediated dispersal could play in spreading future pesticide resistance throughout its Australian distribution.

We thank Aston Arthur for his assistance with collection of samples. We also thank the Department of Food and Agriculture Western Australia and the Department of Primary Industries, Parks, Water and Environment Tasmania for providing samples analysed in this study. This study was supported by the Grains Research and Development Corporation through a graduate studentship, the National Invertebrate Pest Initiative and the Australian Research Council through their Special Research Centre program.

References

- Allendorf, F. W. & Seeb, L. W. (2000). Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution* **54**, 640–651.
- Alvarez, T., Frampton, G. K. & Goulson, D. (1999). The effects of drought upon epigeal Collembola from arable soils. *Agriculture for Entomology* **1**, 243–248.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (2004). In *GENETIX 4.05, Logiciel Sous Windows TM Pour La génétique des Populations* (ed. Laboratoire Génome P, Interactions). Montpellier, France: CNRS UMR 5000, Université de Montpellier II.
- Betsch-Pinot, M. (1976). Reproductive-behaviour of *Sminthurus viridis* (L.) (Collembola, Symphyleona). *Zeitschrift Tierpsychologie* **40**, 427–439.
- Bishop, A. L., Harris, A. M. & McKenzie, H. J. (2001). Distribution and ecology of the lucerne flea, *Sminthurus viridis* (L.) (Collembola: Sminthuridae), in irrigated lucerne in the Hunter dairying region of New South Wales. *Australian Journal of Entomology* **40**, 49–55.
- Bishop, A. L., McKenzie, H. J., Barchia, I. M. & Spohr, L. J. (1998). Efficacy of insecticides against the lucerne flea, *Sminthurus viridis* (L.) (Collembola: Sminthuridae), and other arthropods in lucerne. *Australian Journal of Entomology* **37**, 40–48.

- Conte, R., dos Reis, M., Mantovani, A. & Vencovsky, R. (2008). Genetic structure and mating system of *Eutерpe edulis* Mart. populations: a comparative analysis using microsatellite and allozyme markers. *Journal of Heredity* **99**, 476–482.
- Crawford, N. G. (2010). SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources* **10**, 556–557.
- Davidson, J. (1934). The 'Lucerne Flea' *Smynthurus viridis* L. (Collembola) in Australia. *Bulletin for Council of Scientific and Industrial Research* **79**, 4–69.
- Eckert, C. G., Samis, K. E. & Loughheed, S. C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**, 1170–1188.
- Endersby, N. M., McKechnie, S. W., Ridland, P. M. & Weeks, A. R. (2006). Microsatellites reveal a lack of structure in Australian populations of the diamondback moth, *Plutella xylostella* (L.). *Molecular Ecology* **15**, 107–118.
- Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620.
- Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver 3.0: an integrated software package for population genetics data analysis. *Evolution of Bioinformatics Online* **1**, 47–50.
- Fanciulli, P. P., Melegari, D., Carapelli, A., Frati, F. & Dallai, R. (2000). Population structure, gene flow and evolutionary relationships in four species of the genera *Tomocerus* and *Pogonognathellus* (Collembola, Tomoceridae). *Biological Journal of the Linnean Society of London* **70**, 221–238.
- Fanciulli, P. P., Summa, D., Dallai, R. & Frati, F. (2001). High levels of genetic variability and population differentiation in *Gressittacantha terranova* (Collembola, Hexapoda) from Victoria Land, Antarctica. *Antarctic Science* **13**, 246–254.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.
- Goudet, J. (2001) FSTAT: A Program to Estimate and Test Gene Diversities and Fixation Indices. <http://www2.unil.ch/popgen/softwares/fstat.htm>
- Handler, A. M. & Beeman, R. W. (2003). United States Department of Agriculture – Agricultural Research Service: advances in the molecular genetic analysis of insects and their application to pest management. *Pest Management Science* **59**, 728–735.
- Hartl, D. L. & Clark, A. G. (2007). *Principles of Population Genetics*. Sunderland, MA: Sinauer Associates.
- Hebert, P. & Beaton, M. (1989). *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis: A Practical Handbook*. Windsor, Ontario: Helena Laboratories.
- Hoy, M. (2003). *Insect Molecular Genetics: An Introduction to Principles and Applications*. New York: Academic Press.
- Hoffmann, A. A., Weeks, A. R., Nash, M. A., Mangano, P. G. & Umina, P. A. (2008). The changing status of invertebrate pests and the future of pest management in the Australian grains industry. *Australian Journal of Experimental Agriculture* **48**, 1481–1493.
- Huelsenbeck, J. P. & Andolfatto, P. (2007). Inference of population structure under a Dirichlet process model. *Genetics* **175**, 1787–1802.
- Jost, L. (2008). G(ST) and its relatives do not measure differentiation. *Molecular Ecology* **17**, 4015–4026.
- Klein, C., Rebolledo, R., Cerda, C. & Aguilera, A. (2005). Population density variation of *Sminthurus viridis* (L.) (Collembola: Sminthuridae) in the IX region of La Araucania, Chile. *Gayana* **69**, 260–265.
- Larsson, L. C., Laikre, L., Palm, S., Andre, C., Carvalho, G. R. & Ryman, N. (2007). Concordance of allozyme and microsatellite differentiation in a marine fish, but evidence of selection at a microsatellite locus. *Molecular Ecology* **16**, 1135–1147.
- Lewis, P. O. & Zaykin, D. (2001). Genetic Data Analysis: Computer Program for the Analysis of Allelic Data. <http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php>
- Lushai, G. & Loxdale, H. D. (2004). Tracking movement in small insect pests, with special reference to aphid populations. *International Journal of Pest Management* **50**, 307–315.
- Pescott, R. T. M. (1937). Insect pests of subterranean clover. *Journal of the Department of Agriculture, Victoria* **35**, 371.
- Piry, S., Luikart, G. & Cornuet, J. M. (1999). BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**, 502–503.
- Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
- Richardson, B., Baverstock, P. & Adams, M. (1986). *Allozyme Electrophoresis: A Handbook and Animal Systematics and Population Studies*. Sydney, Australia: Academic Press.
- Roberts, J. M. K., Umina, P. A., Hoffmann, A. A. & Weeks, A. R. (2009). The tolerance of the lucerne flea, *Sminthurus viridis* (Collembola: Sminthuridae), to currently registered pesticides in Australia. *Australian Journal of Entomology* **48**, 241–246.
- Roberts, J. M. K. & Weeks, A. R. (2009). Permanent genetic resources added to molecular ecology resources database 1 May 2009–31 July 2009. *Molecular Ecology Resources* **9**, 1460–1466.
- Robinson, M. T., Weeks, A. R. & Hoffmann, A. A. (2002). Geographic patterns of clonal diversity in the earth mite species *Penthaeus major* with particular emphasis on species margins. *Evolution* **56**, 1160–1167.
- Ryman, N. & Palm, S. (2006). POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* **6**, 600–602.
- Sexton, J. P., McIntyre, P. J., Angert, A. L. & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology Evolution and Systematics* **40**, 415–436.
- Slatkin, M. and Barton, N. H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**, 1349–1368.
- Stevens, M. I. & Hogg, I. D. (2003). Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology* **12**, 2357–2369.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.
- Timmermans, M. J. T. N., Ellers, J., Marien, J., Verhoef, S. C., Ferwerda, E. B. & Van Straalen, N. M. (2005). Genetic structure in *Orchesella cincta* (Collembola): strong subdivision of European populations inferred from mtDNA and AFLP markers. *Molecular Ecology* **14**, 2017–2024.

- Umina, P. A. & Hoffmann, A. A. (1999). Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Australian Journal of Experimental Agriculture* **39**, 621–628.
- Umina, P. A., Hoffmann, A. A. & Weeks, A. R. (2004). Biology, ecology and control of the *Penthaleus* species complex (Acari: Pentheleidae). *Experimental and Applied Acarology* **34**, 211–237.
- van der Wurff, A. W. G., Gols, R., Ernsting, G. & Van Straalen, N. M. (2005). Population structure of *Orchesella cincta* (Collembola; Hexapoda) in NW Europe, as revealed by microsatellite markers. *Pedobiologia* **49**, 167–174.
- Wallace, M. M. H. (1967). The ecology of *Sminthurus viridis* (L.) (Collembola) I. Processes influencing numbers in pastures in Western Australia. *Australian Journal of Zoology* **15**, 1173–1206.
- Wallace, M. M. H. (1968). The ecology of *Sminthurus viridis* (Collembola) II. Diapause in the aestivating egg. *Australian Journal of Zoology* **16**, 871–883.
- Wallace, M. M. H. (1974). Present and probable world distribution of *Sminthurus viridis* and prospects for its biological control. *Pedobiologia* **14**, 238–243.
- Wallace, M. M. H. & Mahon, J. A. (1971). The ecology of *Sminthurus viridis* (Collembola) III. The influence of climate and land use on its distribution and that of an important predator, *Bdellodes lapidaria* (Acari: Bdellidae). *Australian Journal of Zoology* **19**, 177–188.
- Weeks, A. R., Fripp, Y. & Hoffmann, A. A. (1995). Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Pentheleidae). *Experimental and Applied Acarology* **19**, 633–646.
- Weir, B. S. & Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.