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Tracing the geographic origin of the cosmopolitan parthenogenetic insect pest *Liposcelis bostrychophila* (Psocoptera: Liposcelididae)

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Abstract

The randomly amplified polymorphic DNA technique was used to trace the geographic origin of *Liposcelis bostrychophila* Badonnel populations in Australia from unknown geographic sources internationally. Haplotype (or clonal) diversity was high, with 474 unique haplotypes found from 616 individuals genotyped. Gene diversity estimates (0.10–0.28) and percent polymorphic loci (38.1–88.1%) were moderate to high for most populations. This resulted in genetic distance estimates that ranged from 0.04 to 0.26 and were significantly different for most pairwise population combinations. G_{ST} values for all populations were also moderate (0.04–0.54) and again were significantly different for most pairwise population comparisons. Analysis of molecular variance revealed that the majority of variation was apportioned among individuals within populations regardless of the level at which they were grouped. Gene flow (Nm) was mostly low for all pairwise populations comparisons with an average Nm = 1.8. A non-significant negative correlation between genetic distance and geographic distance was found for worldwide populations. In contrast, within Australian populations a significant positive correlation between genetic distance and geographic distance was detected. Genetic relationships explored using unweighted pair group method analysis and non-metric multidimensional scaling indicated a mixed pattern of genetic similarities among all populations. Multiple introductions, from a wide range of international source populations, have obscured the ability to accurately determine the geographic origin of *L. bostrychophila* in Australia.

Keywords: *Liposcelis bostrychophila*, Australia, grain storage, gene flow, geographic origin

Introduction

Liposcelis bostrychophila Badonnel (Psocoptera: Liposcelididae) is a cosmopolitan invasive psocid that has been identified as a major pest of stored grain and their structures in Australia (Rees, 2004) and domestic situations internationally (Baz & Monserrat, 1999; Turner, 1999). Although there is limited information on the dispersal patterns and capabilities of *L. bostrychophila*, they have proven to be a highly invasive species in Australian grain storage systems owing to their obligatory parthenogenetic life history (Yusuf & Turner, 2004) and their ability to withstand significant fluctuations in habitat parameters. Consequently, in Australian grain storage systems low level infestations persist year round. Although there is limited information on the local dispersal pattern and capabilities of *Liposcelis* species, commodity export shipping containers and vessels are a well known mode of worldwide dispersal (Stanaway *et al*.,

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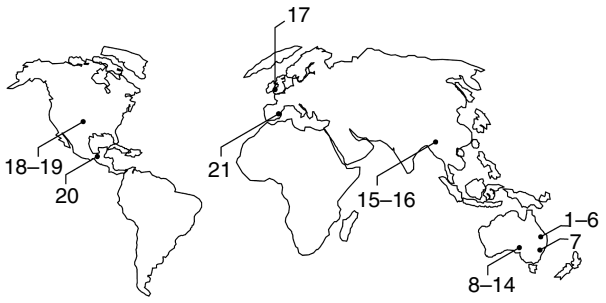

Fig. 1. International locations of the 21 *Liposcelis bostrychophila* populations sampled. Population codes are as follows: 1–7 Qld/NSW; 8–14 SA, 15–16 China; 18–19 USA; 20 Mexico; and 21 Spain.

2001; Kiritani & Yamamura, 2003). In Australia, *Liposcelis* infestations are notorious for contributing to violations of Australian grain export legislation that prescribes a zero tolerance to the export of live insects in grain, resulting in serious legal and monetary ramifications.

Within Australia, grain storage systems can be viewed as an established series of networks of suitable habitat patches. The unintentional movement of individuals within these networks (via trucks, trains and ships) is a key factor in the species persistence, increasing the size of local populations or facilitating the establishment of new populations. In an obligate parthenogen, migration acts to increase the genetic diversity of populations, even though immigrants are not exchanging genes (Vrijenhoek, 1998). The elucidation of genetic structure and variation within and among geographically distinct populations has the potential to provide insights into the geographic origin, migration pathways and colonization history (single or multiple introductions) of the target species.

Randomly amplified polymorphic DNA (RAPD) techniques have proven their utility as a genetic marker with wide applicability in the study of invasive insect species (e.g. Stevens & Wall, 1995; Lou *et al*., 1998; Gasperi *et al*., 2002; Kim & Sappington, 2004), although the technique is known to have limitations (see Hadrys *et al*., 1992 for a review). Such limitations include the irreproducibility of RAPD fragments due to polymerase chain reaction (PCR) sensitivity usually governed by changes in *Taq* polymerase, Mg^{2+} and DNA concentrations. However, these limitations can be overcome by standardizing PCR conditions and enforcing strict quality control measures (Williams *et al*., 1990). In addition, RAPDs are a dominant marker (i.e. homozygotes cannot be distinguished from heterozygotes) and because of this are thought to be less informative than codominant markers. Nevertheless, by genotyping two- to three-fold more individuals it is possible to achieve the same degree of statistical power as codominant markers (Lynch & Milligan, 1994). For small insects such as *L. bostrychophila*, for which there is no nucleotide sequence information and for which only nanogram quantities of DNA can be extracted, PCR-based RAPDs are ideal (see Welsh & McClelland, 1990; Williams *et al*., 1990). Currently, there is no information available regarding the extent and distribution of genetic variation in *L. bostrychophila* populations associated with the grain storage system both in Australia and internationally. In this paper we use RAPDs to assess patterns of genetic diversity, structure and gene flow and investigate whether there is a correlation between geographic and genetic distance of *L. bostrycophila* populations across their current international distribution. Insights into the genetic structure of *L. bostrychophila* populations will be used to investigate the geographic origin of populations associated with Australian grain storage systems.

Materials and methods

Sample collection and processing

A total of 14 *L. bostrychophila* populations were sampled throughout Australia (fig. 1) using crevice traps. *Liposcelis bostrychophila* individuals from seven international populations in five countries (i.e. China, UK, USA, Mexico and Spain) were obtained from colleagues (fig. 1). Total genomic DNA was isolated from whole individual *L. bostrychophila* crushed in 5% Chelex beads (Biorad) following the methods of Walsh *et al*. (1991) and DNA concentrations (i.e. nanograms per microlitre) were quantified by spectrophotometry. Approximately 20 RAPD primers (Operon Technologies-Kit F) of random sequence were screened for variation in *L. bostrychophila*. Three primers were found to be polymorphic (OPF 3, 7 and 9), yielding 42 loci from which to screen for variation in *L. bostrychophila* populations. PCR reaction conditions were carried out in accordance with protocols developed by Williams *et al*. (1990) and Welsh & McClelland (1990). The 25 µl reactions consisted of 0.2 µM dNTPs, 2 mM $MgCl_2$, 1 × PCR reaction buffer, 2 units of *Taq* polymerase (New England Biolabs), 25 ng of DNA and 5 pmols of RAPD operon technology primer. The reaction profile consisted of 40 cycles of 94°C for 1 min, 40°C for 1.5 min and 72°C for 2 min. No initial denaturation or final extension steps were included. Amplifications were carried out on a Corbett Research Palm Thermal Cycler. PCR products were electrophoresed at 80V for 90 min on 1.3% agarose gels and 1 × TBE buffer and visualized using UV light.

Quality control was practised as follows: all water was nano-purified and autoclaved to minimize contamination with non-target DNA that could produce artefacts during PCR. PCR assays were carried out using filter tips to eliminate the possibility of aerosol contamination from carryover products. Fragment size was inferred by comparison with a 2 kb size standard and by running the same two *L. bostrychophila* individuals in each RAPD–PCR assay and subsequently on each gel, as a positive control. Each RAPD–PCR was repeated twice and only reproducible bands found in repeated assays were used in the final data set.

Data analysis

To increase the statistical power of the RAPD technique, as recommended by Lynch & Milligan (1994) and Sjogren & Wyoni (1994), three times as many individuals were used in this study than has been used by others to investigate the genetic population dynamics of invasive insects using RAPDs (e.g. Lou *et al*., 1998). Each individual band/locus was scored for presence (1) or absence (0). Band scoring and gene frequency data was analysed using GelQuant 1.0. Bands that ran at a similar molecular weight were scored as the same band. Each specific RAPD multiband profile was treated as a distinct haplotype (Huff *et al*., 1993) or clone,

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Table 1. Gene diversity, percent polymorphic loci, number of unique haplotypes and frequency of shared haplotypes for Australian and international *Liposcelis bostrychophila* populations.

	Population	H ± S.D.	% P	No. of haplotypes	Frequency of shared haplotypes*
1	Brisbane, Qld ($n = 14$)	0.14 ± 0.02	35.7	4	6(430) 5(447) 2(448)
2	Pittsworth, Qld ($n = 18$)	0.18 ± 0.02	52.3	14	3(473) 2(474)
3	Oakey, Qld ($n = 32$)	0.13 ± 0.16	50.0	24	2(246) 2(296) 2 (298) 2(310) 2(377)
4	Nalla (Toowoomba), Qld ($n = 32$)	0.23 ± 0.19	76.1	20	6(388) 2(390) 3(391) 4(400)
5	Thomby (Dalby), Qld ($n = 32$)	0.19 ± 0.17	80.9	24	4(75) 6(456)
6	Jondaryan, Qld ($n = 32$)	0.17 ± 0.17	64.2	29	2(35) 2(100) 2(174)
7	Temora, NSW ($n = 32$)	0.21 ± 0.18	76.1	27	4(87) 2(330) 2(331)
	Overall	0.24 ± 0.15			
8	Port Adelaide, SA ($n = 8$)	0.10 ± 0.14	42.8	8	0
9	Lock, SA ($n = 32$)	0.14 ± 0.17	61.9	22	6(131) 5(137)
10	Karkoo, SA ($n = 32$)	0.14 ± 0.17	54.7	23	2 (185) 3(121) 2(233) 6(257)
11	Thevenard, SA ($n = 32$)	0.14 ± 0.17	64.2	25	1 (185) 2(108) 2(110) 5(293) 2(294)
12	Redhill, SA ($n = 32$)	0.15 ± 0.19	52.3	20	6(105) 4(209) 2(269) 4(305)
13	Alawoona, SA ($n = 32$)	0.12 ± 0.19	38.1	13	8(17) 2(107) 4(302) 2(308)
14	Cowell, SA ($n = 32$)	0.13 ± 0.16	61.9	29	2(181) 2(187) 2(242)
	Overall	0.19 ± 0.19			
15	China 1 ($n = 32$)	0.18 ± 0.18	71.4	32	0
16	China 2 ($n = 32$)	0.28 ± 0.17	88.1	31	3(444)
17	UK ($n = 32$)	0.19 ± 0.18	69.0	24	7(32) 3(346)
18	USA 1 ($n = 32$)	0.12 ± 0.17	45.2	23	2(91) 2(140) 3(253) 2(381)
19	USA 2 ($n = 32$)	0.18 ± 0.18	66.6	31	2(273)
20	Mexico ($n = 32$)	0.12 ± 0.17	47.6	21	4(226) 2(86) 3(136) 3(141) 2(143)
21	Spain ($n = 32$)	0.16 ± 0.17	78.5	32	0
	Overall	0.22 ± 0.15			

n, sample size; H, Nei's (1973) gene diversity; S.D, standard deviation; %P, percent polymorphic loci; *, frequency of shared haplotypes within populations, haplotype number shown in parentheses.

given that *L. bostrychophila* is parthenogenetic. Haplotype frequency estimation and number of haplotypes common among populations was calculated using GenAlEx (Peakall & Smouse, 2006). Nei's (1973) gene diversity (H), precent polymorphic loci and pairwise Nei's (1978) genetic distance (D) estimates and the significance of individual population pairwise comparisons were evaluated using Bartlett's test of homogeneity of RAPD variance in POPGEN (Yeh *et al.*, 1997). G_{ST} was used to estimate population structure using TFPGA (10,000 permutations, Miller, 1997). Significance tests for genetic differentiation among populations were based on exact tests following the procedures of Raymond & Rousset (1995) and corrected for multiple tests using the Bonferroni procedure (10,000 permutations, Rice, 1989).

Partitioning of genetic structure with regard to geographic scale was assessed by analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using the GenAlEx program to estimate Φ_{ST} (9999 permutations, Peakall & Smouse, 2005). Total genetic variation was partitioned into three levels: (i) among geographic regions (worldwide and within Australia); (ii) among populations within geographic regions; and (iii) among individuals within populations. A total of two AMOVAs were conducted. The first AMOVA was conducted for worldwide populations that were subdivided into four geographic regions: region 1, all Australian populations; region 2, China 1 and 2; region 3, UK and Spain; and region 4: USA 1, 2 and Mexico. The second AMOVA was performed for Australian populations only, with two geographic regions identified: region 1, Queensland (Qld) and New South Wales (NSW) populations combined; and region 2, South Australian (SA) populations combined. Whether a correlation existed between Nei's unbiased (1978) genetic distance (D) and the geographic distance between populations was assessed with a Mantel test using TFPGA (Miller, 1997). Gene flow (N*m*) was estimated for the RAPD data from G_{ST} by the formula for number of migrating females: $Nm_f = 0.5\ (1/F_{ST} - 1)$ (Slatkin, 1995), this formula being appropriate given that *L. bostrychophila* is an obligate parthenogenetic species. Pairwise measures of Nei's (1978) genetic distance among all 21 populations were used in an unweighted pair group method (UPGMA) cluster analysis to construct a dendrogram of genetic affinity among populations using TFPGA (Miller, 1997). Further exploration of the genetic relationships among populations was undertaken using haplotype frequency estimates visualized by non-metric multidimensional scaling (nMDS) using PRIMER V5.0 (Clarke & Gorley, 2001).

Results

Haplotype frequency, genetic diversity and distance estimates

A total of 474 unique haplotypes or clones were found among the 616 samples. International populations had a range of 21–32 haplotypes and Australian populations had between 4–29 haplotypes per population (table 1). Only Karkoo, SA and Thevenard, SA shared a single haplotype, the remaining haplotypes were all unique to a specific population (table 1). The frequency of shared haplotypes within populations ranged from 0 to 5 resulting in a high percentage of alleles only observed once (table 1). Individual population genetic diversity estimates were high (table 1). Nei's (1973) gene diversity (H) ranged from 0.12 to 0.23 and % polymorphic loci ranged from 38.1 to 88.1% respectively (table 1). Overall group estimates for H and % polymorphic

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Table 2. Matrix of approximate linear geographic distances (km) (above diagonal) and Nei's (1978) genetic distance (D) (below diagonal) for all populations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1		140	129	106	177	142	935	1604	1784	1803	1936	1568	1437	1686	8060	8060	16409	13846	13846	12709	16940
2	0.14		33	36	70	39	843	1475	1649	1669	1798	1435	1312	1552	8010	8010	16367	13978	13978	12848	16843
3	0.24	0.18		27	53	15	875	1501	1671	1691	1817	1458	1339	1575	7988	7988	16343	13955	13955	12835	16831
4	0.22	0.15	0.25		78	41	873	1511	1685	1705	1833	1471	1348	1588	8011	8011	16366	13941	13941	12815	16857
5	0.14	0.12	0.06	0.17		38	882	1482	1645	1667	1786	1435	1323	1550	7939	7939	16295	13978	13978	12874	16777
6	0.25	0.18	0.07	0.22	0.07		876	1495	1663	1684	1807	1450	1333	1567	7975	7975	16330	13962	13962	12847	16816
7	0.23	0.18	0.06	0.17	0.07	0.08		826	1089	1085	1314	863	643	980	8460	8460	16767	14669	14669	13326	16889
8	0.16	**0.10**	0.14	0.17	**0.08**	0.10	0.10		290	270	541	148	183	194	8156	8156	16242	15166	15166	14143	16155
9	0.27	0.20	0.09	0.18	0.10	0.08	0.04	**0.08**		52	252	229	456	109	7937	7937	15982	15627	15627	14411	15866
10	0.21	0.14	0.06	0.19	0.07	0.06	0.06	**0.07**	0.06		285	237	445	117	7878	7878	16017	15649	15649	14413	15885
11	0.28	0.21	0.05	0.25	0.07	**0.04**	0.05	0.12	0.06	**0.05**		454	698	350	7725	7725	15732	15744	15744	14612	15619
12	0.23	0.20	0.06	0.23	0.10	0.05	0.09	0.13	0.11	0.06	0.07		262	124	8012	8012	16133	15415	15415	14182	16070
13	0.24	0.25	0.14	0.25	0.16	0.10	0.16	0.19	0.18	0.12	0.13	0.05		350	8214	8214	16373	15273	15273	13972	16317
14	0.26	0.23	0.12	0.23	0.12	0.07	0.10	0.11	0.10	0.05	0.08	0.05	0.08		7985	7985	16064	15532	15532	14303	15969
15	0.20	0.09	0.13	0.14	0.09	0.10	0.08	**0.07**	0.09	0.08	0.12	0.13	0.18			5	8330	11318	11318	13054	9030
16	0.18	0.15	0.15	0.10	0.12	0.15	0.10	0.14	0.11	0.10	0.14	0.14	0.22	0.15			8330	11318	11318	13054	9030
17	0.19	0.16	0.09	0.21	0.06	0.08	0.08	0.11	0.09	0.10	0.06	0.10	0.15	0.15	0.09	0.14		6743	6743	8794	1419
18	0.26	0.19	0.05	0.21	0.05	0.07	0.06	0.09	0.05	0.07	0.05	0.10	0.18	0.11	0.10	0.14	0.08		10	2266	7660
19	0.21	0.18	0.09	0.22	0.10	0.07	0.09	0.14	0.12	0.07	0.07	**0.02**	0.06	0.13	0.09	0.15	0.09	0.13		2266	7660
20	0.26	0.19	0.06	0.20	0.07	0.04	0.06	0.10	0.07	0.04	**0.04**	0.08	0.12	0.05	0.13	0.13	0.11	0.06	0.09		9487
21	0.22	0.18	0.16	0.23	0.10	0.15	0.13	0.16	0.15	0.12	0.13	0.17	0.18	0.07	0.09	0.15	0.09	0.14	0.15	0.15	

Most populations were significant after corrections for multiple tests ($P < 0.05$). The nine non-significant genetic distance values are indicated in bold. Population codes are listed in table 1.

loci were greatest for the Qld and NSW populations, intermediate for the seven international populations and lowest in the SA populations (table 1). Genetic distances among populations ranged from 0.005 to 0.21, with an average of 0.08 (table 2). Exact tests of significance of D estimates indicated significant ($P < 0.05$) differences for most pairwise population comparisons after correction for multiple tests ($n = 210$, table 2). Within and between regional groups estimates for D did not greatly vary based on geographic scales and ranged from 0.06 to 0.09 (table 3).

Genetic structure

Pairwise estimates of G_{ST} for all populations ranged from 0.04 to 0.54 with an average value of 0.28 (table 4). G_{ST} estimates were significant ($P < 0.01$) for most pairwise estimates, after correction for multiple comparisons ($n = 210$). The non-significant G_{ST} estimates were between Alawoona, SA and Dalby, Qld and Redhill, SA and Alawoona, SA (table 4). Similarly, G_{ST} estimates were moderately high overall for the international populations ($G_{ST} = 0.28$; $P < 0.01$), and similar to the overall G_{ST} among SA populations ($G_{ST} = 0.20$; $P < 0.01$) and the Qld and NSW populations ($G_{ST} = 0.26$; $P < 0.01$). G_{ST} estimates within and between different regions at differing geographic scales varied little and ranged from 0.24 to 0.29 (table 3). The two AMOVA analyses conducted indicated that the majority of genotypic variation (>70%) was highly significant and apportioned among individuals within geographic regions both worldwide (% variation = 72; $P < 0.001$) and within Australia (% variation = 70; $P < 0.001$). The remaining AMOVA variation was distributed among populations within geographic regions at the worldwide (% variation = 26; $P < 0.001$) and Australia level (% variation = 26; $P < 0.001$). Pairwise estimates of gene flow (Nm) among all populations ranged from 0.42 to 10.61 with an average of 1.8 (table 3). Average Nm values were similar among Qld and NSW (Nm = 2), SA (Nm = 1.43) and international (Nm = 1.28) populations. Nm estimates among regions were similar regardless of geographic scale and ranged from 1.13 to 1.57 (table 3). A Mantel test revealed a negative non-significant correlation between geographic and genetic distance among worldwide populations ($r^2 = -0.12$; $P = 0.81$). In contrast, among Australian populations a significant positive correlation was found between geographic and genetic distance ($r^2 = 0.11$; $P < 0.05$).

UPGMA based genetic relationships among populations indicated a lack of geographic concordance with genetic distance (fig. 2). Within six major clusters formed, Qld/NSW populations were placed in four of them, SA populations were within three of the clusters and international populations that are closest geographically did not cluster together (i.e. USA 1 and 2, China 1 and 2, Spain and UK: fig. 2). Every cluster with more than one population includes Australian populations clustered with at least one international population (fig. 2). Additional examination of genetic relationships among populations using nMDS revealed a single compact cluster consisting of many populations from most regions surrounded by a more distant affiliation of populations from each region (fig. 3). One obvious outlier was the population at Port Adelaide, SA (fig. 3). Except for the single outlier, the populations from SA were grouped more closely than those from Qld/NSW (fig. 3).

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Table 3. Nei's (1978) genetic distance (D), genetic structure (G_{ST}) and gene flow (N*m*) within and between populations for predefined geographic scales.

Population	D	G_{ST}	N*m*
Qld/NSW (within)	0.09	0.26	1.35
Qld/NSW vs. SA	0.09	0.30	1.13
Qld/NSW vs. China	0.08	0.24	1.57
Qld/NSW vs. USA/Mexico	0.08	0.27	1.32
Qld/NSW vs. Spain/UK	0.08	0.26	1.39
Qld/NSW vs. International	0.07	0.25	1.49
SA (within)	0.09	0.28	1.22
SA vs. China	0.07	0.26	1.39
SA vs. USA/Mexico	0.06	0.27	1.31
SA vs. Spain/UK	0.07	0.29	1.21
SA vs. International	0.07	0.26	1.40

Discussion

To determine the geographic origin of *L. bostrychophila* populations in Australia, genetic diversity estimates were used as indicators of source populations. Source populations retain higher levels of genetic diversity compared to recently founded populations, which have reduced genetic diversity, as demonstrated by Gasperi *et al*. (2002) for the invasive medfly, *Ceratitis capitata* (Wiedemann). In this study, genetic diversity estimates (H and % polymorphic loci) were highest for the China 2 population and in addition high haplotype or clonal diversity was also found (table 1). However, in the absence of genetic information from highly variable co-dominant markers such as microsatellites there is insufficient evidence to implicate China as a geographic source of contemporary populations associated with Australian grain storage systems. Nevertheless within Australia, populations from Queensland and New South Wales also displayed high haplotype/clonal and genetic diversity estimates and are the most obvious source of dispersing individuals that subsequently colonized and established populations elsewhere within Australia (tables 1, 2, 3 and 4). Supporting evidence comes from the first documented reports early last century of *Liposcelis* species causing economic losses to grain storages in Queensland (Caldwell, 1947; Champ & Smithers, 1965). The unintentional movement of individuals within grain storage networks (via trucks, trains and ships) is a key factor in the persistence of *L. bostrychophila* within Australian grain storage systems (table 4), as shown by the Karkoo and Thevenard populations in SA that are connected via a single rail network and shared a common haplotype (table 1).

This study provides the first evidence of genetic structure throughout the cosmopolitan distribution of *L. bostrychophila* populations. It has often been speculated and since shown (Stanaway *et al*., 2001) that *L. bostrychophila* individuals disperse via human mediated routes. Owing to the mobility of *L. bostrychophila* individuals worldwide

Table 4. Matrix of pairwise N*m* ($Nm_f = 0.5\ (1/F_{ST} - 1)$) (above diagonal) and G_{ST} estimates with exact test difference (below diagonal) for all populations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1		0.89	1.12	1.33	0.63	0.45	0.50	0.42	0.47	0.81	0.50	0.61	0.81	0.78	1.35	0.90	0.47	0.44	0.77	0.69	0.56
2	0.35		1.23	1.25	0.97	0.75	1.10	0.61	0.74	1.48	0.70	0.92	1.21	1.60	2.87	1.13	0.86	0.84	0.93	0.92	0.80
3	0.30	0.28		1.71	1.10	0.75	0.77	0.46	0.72	1.17	0.76	0.96	1.68	2.17	1.61	1.21	0.74	0.71	0.97	0.96	0.75
4	0.27	0.28	0.22		2.35	1.29	1.73	0.85	2.34	3.07	2.78	2.72	2.83	2.10	2.14	3.63	3.12	2.13	2.01	1.83	1.72
5	0.44	0.33	0.31	0.17		2.27	2.39	0.85	2.78	3.09	2.00	2.72	2.35	1.90	2.10	1.62	1.81	2.92	2.17	1.15	2.14
6	0.52	0.39	0.39	0.27	0.18		1.24	1.21	1.54	1.61	0.92	1.98	1.04	0.88	1.29	1.26	0.87	1.46	3.12	0.83	2.78
7	0.49	0.31	0.39	0.22	0.17	0.28		0.59	2.07	4.21	1.28	1.92	1.81	1.59	1.92	1.88	2.56	1.78	1.21	0.96	1.20
8	0.54	0.44	0.51	0.36	0.36	0.29	0.45		0.83	0.88	0.67	1.20	0.49	0.67	0.83	0.84	0.54	0.80	2.16	0.65	2.07
9	0.51	0.40	0.40	0.17	0.15	0.24	0.19	0.37		3.84	2.39	3.88	1.11	1.20	1.46	2.87	2.39	3.31	2.03	1.14	1.95
10	0.38	0.25	0.29	0.14	0.13	0.23	0.10	0.36	0.11		2.45	2.47	2.14	2.37	2.99	2.68	2.90	2.53	2.24	1.46	1.85
11	0.50	0.41	0.39	0.15	0.20	0.35	0.28	0.42	0.17	0.16		2.07	0.84	1.08	1.36	1.58	2.32	1.95	1.58	0.86	1.95
12	0.44	0.35	0.34	0.15	0.15	0.20	0.20	0.29	0.11	0.16	0.19		1.81	1.71	1.60	2.27	2.07	3.88	2.44	1.09	2.83
13	0.38	0.29	0.22	0.15	**0.17**	0.32	0.21	0.50	0.31	0.18	0.37	**0.21**		2.68	2.01	1.81	1.45	1.21	1.24	0.99	1.01
14	0.38	0.23	0.18	0.19	0.20	0.36	0.23	0.42	0.29	0.17	0.31	0.22	0.15		2.94	1.77	1.47	1.41	1.27	1.41	1.16
15	0.26	0.14	0.23	0.18	0.19	0.27	0.20	0.37	0.25	0.14	0.26	0.23	0.19	0.14		1.85	1.44	1.49	1.62	1.54	1.49
16	0.35	0.30	0.29	0.12	0.23	0.28	0.21	0.37	0.14	0.15	0.24	0.18	0.21	0.22	0.21		2.02	1.26	1.92	1.98	1.59
17	0.51	0.36	0.40	0.13	0.21	0.36	0.16	0.48	0.17	0.14	0.17	0.19	0.25	0.25	0.25	0.19		1.77	1.03	1.01	1.15
18	0.52	0.37	0.41	0.19	0.14	0.25	0.21	0.38	0.13	0.16	0.20	0.11	0.29	0.26	0.25	0.28	0.22		1.43	0.87	1.53
19	0.39	0.34	0.33	0.19	0.18	0.13	0.29	0.18	0.19	0.18	0.24	0.17	0.28	0.28	0.23	0.20	0.32	0.25		1.08	10.61
20	0.41	0.35	0.34	0.21	0.30	0.37	0.34	0.43	0.30	0.25	0.36	0.31	0.33	0.26	0.24	0.20	0.33	0.36	0.31		0.85
21	0.46	0.38	0.39	0.22	0.18	0.15	0.29	0.19	0.20	0.21	0.20	0.15	0.33	0.30	0.25	0.23	0.30	0.24	0.04	0.36	

Nearly all G_{ST} estimates were highly significant ($P < 0.001$) after corrections for multiple comparisons. Non-significant G_{ST} values are indicated in bold. Population codes are listed in table 1.

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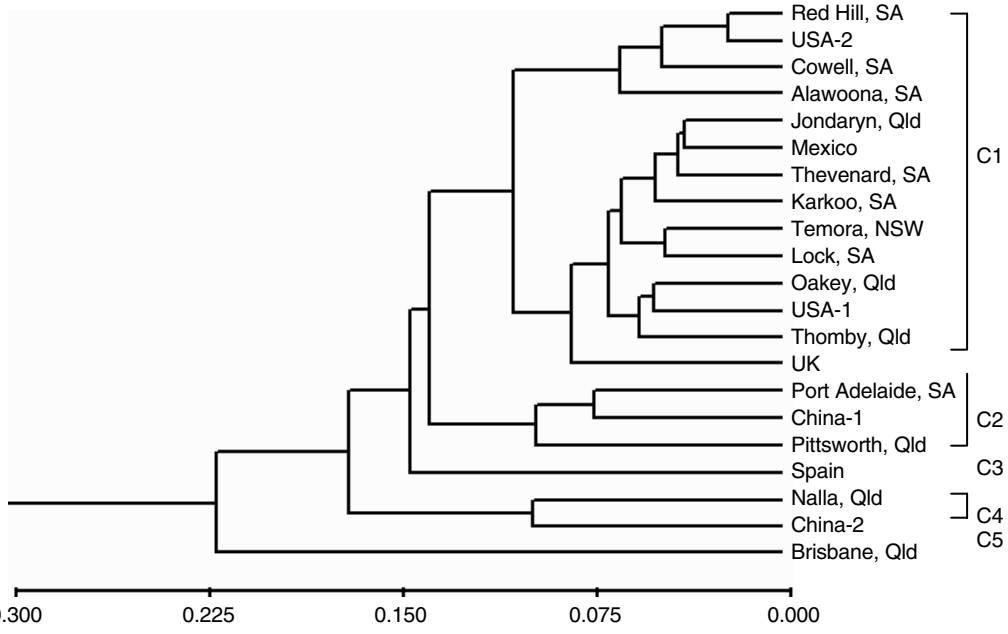

Fig. 2. Unweighted pair group method phenogram based on Nei's (1978) genetic distance showing relationships among Australian and International populations of *Liposcelis bostrychophila*. Branch lengths represent genetic distance. C 1 = cluster 1, etc.

(Stanaway *et al*., 2001; table 4), it is likely that unknown historic colonization and extinction processes have greatly influenced the contemporary genetic structure of worldwide populations investigated here. This is supported by G_{ST} estimates within and between geographic regions that were generally moderate causing some divergence among populations worldwide (table 4). The high intrapopulation variation found for the four predefined geographic regions (>70%) suggests that despite generally moderate gene flow estimates *L. bostrychophila* is mobile enough to move between geographic locations indiscriminately and maintain high genetic diversity within populations worldwide (table 4). Worldwide, gene flow is likely to be multidirectional as no relationship between genetic and geographic distance was found (tables 3 and 4). Evidence of multidirectional gene flow among worldwide *L. bostrychophila* populations was also supported by UPGMA and nMDS analyses that both indicated mixed patterns of population genetic similarities with no clear geographic based patterns visible (figs 2 and 3). In contrast, within Australia a pattern of isolation by distance is likely to be operating as indicated by a weak positive, yet significant relationship between geographic and genetic distance. These findings suggest that although within Australia *L. bostrychophila* gene flow is reduced, worldwide gene flow is moderate, such that Australia has been subject to multiple introduction of *L. bostrychophila* individuals.

The high levels of genetic and haplotype diversity and moderate G_{ST} and N*m* estimates found within and among *L. bostrychophila* populations in this study contrast with RAPD genetic estimates found for sexually reproducing invasive insects such as the medfly *C. capitata* (Gasperi *et al*., 2002) and the boll weevil *Anthonomus grandis* Boheman (Kim & Sappington, 2004). However, our findings are similar to those of Lou *et al*. (1998) for the parthenogenetic (arrhenotokous) wheat stem sawfly *Cephus cinctus* Norton and an allozyme investigation of *L. bostrychophila*

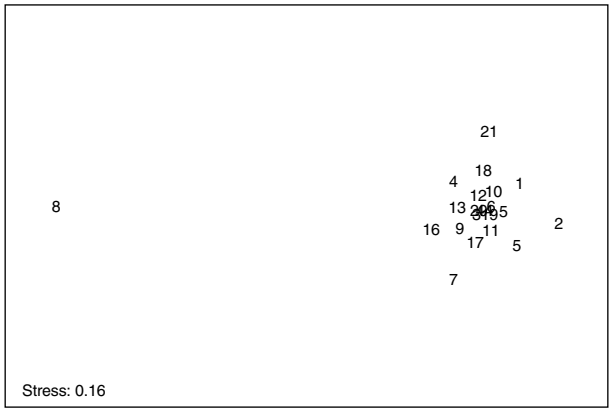

Fig. 3. Non-metric multidimensional scaling of haplotype frequencies for the 21 *Liposcelis bostrychophila* populations investigated. Population codes are as follows; 1–7 Qld/NSW; 8–14 SA, 15–16 China; 18–19 USA; 20 Mexico; and 21 Spain.

populations from the UK that showed significant interpopulation variability (Ali & Turner, 2001). *Liposcelis bostrychophila* infestations associated with grain storage systems in Australia are characterized by large fluctuations in population size constantly changing due to colonization and extinction rates of subpopulations (see Ray, 2001). Effective population size (N_e) is considered to be important to the maintenance of genetic variation regardless of an organisms' life history. Indeed, it is thought that as a consequence of genetic drift and or inbreeding small populations should show lower levels of genetic variability than large populations as shown for *L. bostrychophila* in this study. Small populations are therefore less capable of adapting to novel stochastic habitats, as a result of the loss

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of adaptive or potentially adaptive genetic variation through genetic drift (Reed *et al*., 2003). Indeed, parthenogenesis has been linked to individuals having a broad tolerance to environmental and habitat stresses (Vrijenhoek, 1998), thus allowing individuals to colonize and persist in marginal or highly heterogenous environments such as grain storage systems in Australia. Further, reproduction efficiency in parthenogenetic organisms is two-fold greater because there is no need to secure a mate to reproduce (Vrijenhoek, 1998; Simon *et al*., 2003). It thus can be argued that the parthenogenetic life history of *L. bostrychophila* is advantageous to its invasion success worldwide, particularly as parthenogenetic or essentially clonal diversity can be high (Lou *et al*., 1998; Ali & Turner, 2001) suggesting that parthenogenetic species can evolve in response to changing environmental conditions. The present study has shown that despite being an obligate parthenogenetic species, *L. bostrychophila* populations retain high levels of genetic and haplotype diversity possibly affording individuals advantages during each phase of the invasion process (i.e. arrival, colonization and establishment).

Through its successful human-mediated dispersal and unrestricted reproductive potential, *L. bostrychophila* has become one of the most important pests of grain storage systems in Australia. Future work will concentrate on the development and use of highly variable microsatellite markers that will be used to decipher the fine scale international dispersal patterns of this species. Assessing pathways of introduction and geographic origin of *L. bostrychophila* will improve integrated pest management strategies and determine the current and continued risk such invasive insects pose to the biosecurity of Australia.

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