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High levels of genetic variability and population differentiation in *Gressittacantha terranova* (Collembola, Hexapoda) from Victoria Land, Antarctica

PIETRO PAOLO FANCIULLI, DOMENICO SUMMA, ROMANO DALLAI and FRANCESCO FRATI*

Department of Evolutionary Biology, University of Siena, via P.A. Mattioli 4, 53100 Siena, Italy
**corresponding author - frati@unisi.it*

Abstract: Allozyme electrophoresis was used to assess genetic variability and differentiation in 22 populations of *Gressittacantha terranova* Wise (Hexapoda, Collembola) from a coastal area of Victoria Land between the Mariner Glacier and the Nansen Ice Sheet. Allelic frequencies were determined at five enzyme loci: *Phi*, *Pgm*, *Hk*, *Mpi* and *Mdh*. Levels of variability, estimated as rates of heterozygosity, were higher than those calculated for the same loci in taxonomically related and non-related species of non-Antarctic Collembola. Thus, in spite of the ecological simplicity of Antarctic terrestrial ecosystems, *G. terranova* is characterized by high levels of genetic variability, and the 22 populations could be divided into three geographic groups, separated by the Aviator and Campbell glaciers. Genetic differentiation reflects the geographic arrangement of the populations, suggesting that the glaciers are effective barriers to gene flow, and that the patchy distribution of collembolan species in Antarctica has the potential to induce, in the long term, microspeciation processes. Interestingly, detectable genetic differentiation was observed between six populations collected at Edmonson Point, even though these are very close to each other, indicating the impact of geographic isolation even within short distances. The only exception to the congruence between genetic and geographic structuring was provided by the population of Apostrophe Island, for which a recent introduction with individuals coming from southern populations is suggested.

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Key words: allozyme electrophoresis, gene flow, geographic differentiation, genetic distance, heterozygosity, speciation

Introduction

Gressittacantha terranova Wise, 1967 (Hexapoda, Collembola, Isotomidae) is endemic to Victoria Land, and is one of only eleven species of Collembola reported from Eastern (=Continental) Antarctica (Greenslade 1995). This species is the only known representative of the genus *Gressittacantha* (Wise 1967, Dallai *et al.* 1986). The range of the species includes the Northern Foothills of Victoria Land (Wise 1967), with limits of distribution at Football Saddle (72°30'S, 169°42'E) in the north, and Tripp Island (76°38'S, 162°42'E) in the south (Frati *et al.* 1997, Frati 1999). *Gressittacantha terranova* is very rare at its geographical limits, where *Isotoma klovstadi* Carpenter, 1908 and *Gomphiocephalus hodgsoni* Carpenter, 1908, respectively, are dominant. *Gressittacantha terranova* is the dominant species between the Mariner Glacier (73°20'S) and the Nansen Ice Sheet (74°55'S) (Fig. 1) where it usually outnumbers the sympatric species *Friesea grisea* (Schäffer, 1891) (Frati *et al.* 1997).

The development of collembolan communities in Antarctica, as with all Antarctic soil arthropods (Kennedy 1993, Block 1994), is largely dependent upon the availability of free water, the presence of some vegetation, a direct or indirect food source (Kennedy 1999), and stones which provide shelter from dehydrating winds (Frati *et al.* 1997). When these conditions are met, *G. terranova* occurs in large numbers under stones. Such conditions are not uniformly distributed, and may vary drastically even within short distances, thus ensuring collembolan populations are patchy. Furthermore, larger geographic obstacles, for example glaciers, are geographic barriers which effectively confine populations, and limit gene flow even between adjacent populations, promoting differentiation and even microspeciation processes (Nei *et al.* 1983).

Several genetic markers can be used to assess the levels of genetic variability and differentiation within and between natural populations (Frati *et al.* 2000, Gaffney 2000, Medlin *et al.* 2000, Skotnicki *et al.* 2000). Allozyme loci are perhaps the most efficient and widely exploited marker used to study the differentiation between closely related species or conspecific populations, and assess levels of genetic variability. Allozymes have also been extensively used to study population genetics, species boundaries and gene flow in collembolan species from Italy and Europe (Frati *et al.* 1992, Carapelli *et al.* 1995, 1997, Fanciulli *et al.* 2000), studies which provide a large amount of comparative data for Antarctic taxa.

To test whether the challenging Antarctic environment induces high levels of inter-population genetic differentiation,

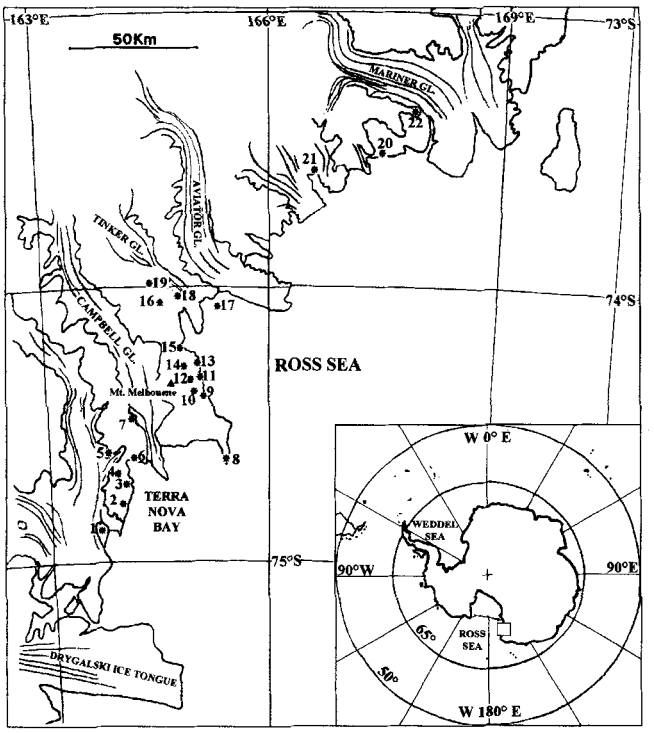

Fig. 1. Map of collecting sites of the 22 populations. See Table I for the number indicating each population.

Table I. List of populations studied in this work. See Fig. 1 for their geographic location.

Population	code	coordinates
1. Inexpressible Island	INXP	74°54'S, 163°42'E
2. Adélie Cove	ADCV	74°46'S, 164°07'E
3. "Springtail valley"	SPTV	74°43'S, 164°07'E
4. Northern Foothills	NRTF	74°43'S, 164°05'E
5. Cape Sastrugi	CPST	74°37'S, 163°40'E
6. Gondwana station	GODW	74°38'S, 164°14'E
7. Campbell Glacier	CPGL	74°26'S, 164°10'E
8. Cape Washington	CPWT	74°38'S, 166°25'E
9. Edmonson Point, "Lake Top hill" (LTH)	EPLT	74°19'S, 165°03'E
10. Edmonson Point, north	EPNT	74°18'S, 165°03'E
11. Edmonson Point, beach	EPBC	74°20'S, 165°05'E
12. Edmonson Point, north valley (NV)	EPNV	74°19'S, 165°05'E
13. Edmonson Point, microclimate station (MCS)	EPMC	74°20'S, 165°04'E
14. Edmonson Point, south	EPSO	74°22'S, 165°05'E
15. Baker Rocks	BKRK	74°14'S, 164°50'E
16. Mount McGee	MMGE	74°04'S, 164°33'E
17. Kay Island	KAYI	74°05'S, 165°20'E
18. Tinker Glacier	TKGL	73°57'S, 165°34'E
19. Burns Glacier	BUGL	73°56'S, 164°11'E
20. Apostrophe Island	APTI	73°31'S, 167°26'E
21. Cape King	CPKG	73°35'S, 166°38'E
22. Emerging Island	EMGI	73°23'S, 168°03'E

we report on the allele frequencies observed at five enzyme loci in individuals from populations of *Gressittacantha terranova* collected in the Victoria Land in 1995–96 and 1998–99. This represents the first study on population genetics of Antarctic soil microarthropods, previous studies having focused on marine organisms (Fevolden & Schneppenheim 1989, Brierley & Thorpe 1994, Yokawa 1994, Duhamel *et al.* 1995, Bargelloni *et al.* 2000, Medlin *et al.* 2000), mosses (Skotnicki *et al.* 2000), and nematodes (Courtright *et al.* 2000).

Materials and methods

Specimens of *G. terranova* were collected from 22 geographically isolated populations (Table I) between the Mariner Glacier and the Nansen Ice Sheet (Fig. 1), reached via helicopters based at Terra Nova Bay Research Station. Six of these populations were collected at Edmonson Point during the 1995–96 BIOTEX-1 camp of the BIOTAS project (Smith & Wynn-Williams 1992). Even the six populations from Edmonson Point may be considered geographically isolated from each other, as they were separated by barren or ice-covered ground. Individual springtails were collected with a mechanical aspirator and/or extracted from soil samples using Tullgren funnels, sorted in the laboratory, killed in liquid nitrogen and stored at -80°C until used for electrophoresis.

Cellulose-acetate electrophoresis (Richardson *et al.* 1986) was done in a Tris-Glycine buffer (0.025M Tris, 0.192M glycine, pH 8.2), with gel-plates allocating 12 lanes. Single specimens were individually ground in 8 µl of distilled water and each lane of the gel was loaded with the homogenate of one individual. Each specimen provided enough material to load two gel-plates, each one stained for a different enzyme. After a 30–40 min run at 200 V, gels were stained with different staining mixtures according to the target enzymes (Murphy *et al.* 1996). A total of five loci, encoding for five different enzymes, gave interpretable banding patterns: Hexokinase (*Hk*; EC: 2.7.1.1), Mannose phosphate isomerase (*Mpi*; EC: 5.3.1.8), Phosphohexoso isomerase (*Phi*; EC: 5.3.1.9), Phosphoglucomutase (*Pgm*; EC: 5.4.2.2) and Malate dehydrogenase (*Mdh*; EC: 1.1.1.7). All these enzymes participate in fundamental metabolic pathways, and the possibility of contamination from the food is negligible due to the much higher amount of enzyme which is provided from the animal's cells. Additional loci were tested (*Ark*, *G3pdh*, *Got*, *Hbdh* and *Idh*) but they did not provide consistent banding patterns, possibly as a consequence of small specimen size (1.0–1.4 mm).

Gels were scored immediately after staining and letters (A, B, C, D, ...) were assigned to each allele with increasing distance from the origin. Observed genotype frequencies were entered in the software BIOSYS-1 (Swofford & Selander 1981) which was used to calculate allele frequencies at each population. The same program allowed the assessment of within-population variability by estimating observed and expected (under the Hardy-Weinberg equilibrium) heterozygosity, mean number of alleles per locus and percentage of polymorphic loci. Between-population

Table II. Allelic frequencies observed in the 22 populations at the five loci studied. Estimates of heterozygosity averaged across all loci for each population are also provided. *n* = number of individual sampled (i.e. number of genotypes scored) for each locus in each population, He = expected heterozygosity under the Hardy-Weinberg equilibrium, Ho = observed heterozygosity.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	INXP	ADCV	SPTV	NRTF	CPST	GODW	CPGL	CPWT	EPLT	EPNT	EPBC	EPNV	EPMC	EPSO	BRRK	MMGE	KAYI	TKGL	BUGL	APTI	CPKG	EMGI
Phi																						
n	22	46	26	21	27	39	26	16	23	16	119	33	43	28	21	31	94	47	17	32	46	43
A	.023	.022	.077	-	.019	-	-	-	-	-	.013	-	-	.018	-	-	.011	.021	.029	-	-	.070
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.016	-	-	-	-	-	-
C	.273	.261	.346	.190	.259	.256	.212	.219	.130	.031	.181	.061	.186	.018	.143	.113	.117	.021	.265	.172	.054	.302
D	.091	.478	.250	.119	.278	.397	.442	.031	.348	.188	.185	.394	.291	.500	.262	.387	.500	.489	.324	.422	.424	.558
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.005	-	-	-	-	-
F	.591	.239	.288	.524	.259	.308	.346	.750	.522	.781	.601	.545	.523	.446	.524	.484	.367	.468	.382	.328	.478	.023
G	.023	-	.038	.167	.185	.038	-	-	-	-	.021	-	-	.018	.071	-	-	-	-	.078	.043	.047
Pgm																						
n	31	26	23	26	39	53	22	16	21	15	130	32	38	24	28	15	95	20	26	24	63	39
A	-	.212	.043	-	-	.066	-	-	-	-	-	-	-	-	-	-	-	-	.077	-	-	.077
B	-	-	.087	.019	.269	.340	-	-	.048	-	-	-	-	-	.018	-	-	.050	-	-	-	-
C	.532	.500	.217	.442	.397	.302	1.000	.125	.238	.200	.258	.063	.158	.104	.107	.367	.453	.525	.154	.125	.944	.910
D	.323	-	.391	.404	.295	.189	-	.156	.071	-	.092	.172	.145	.167	.143	.367	.242	.175	.500	.146	.032	-
E	-	-	-	-	-	.075	-	-	.048	.200	.092	.156	.132	.104	.071	.067	.021	-	.038	.104	.008	-
F	.145	.288	.261	.135	.026	.028	-	.688	.357	.300	.458	.484	.408	.396	.429	.200	.205	.175	.058	.604	.016	.013
G	-	-	-	-	-	-	-	.031	.167	.233	.073	-	.105	.146	.107	-	.068	.025	.115	.021	-	-
H	-	-	-	-	.013	-	-	-	.071	.067	.027	.125	.053	.083	.125	-	.011	.050	.058	-	-	-
Mpi																						
n	15	28	20	34	47	67	9	16	22	22	52	24	20	15	24	20	19	25	9	20	28	33
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.050	-	-	-	-	-	-
B	-	-	-	-	-	-	-	.844	.955	.932	.875	.938	.900	.867	.708	.950	.947	.940	.833	1.000	-	-
C	-	-	-	.015	-	-	-	.156	.045	.068	.125	.063	.100	.133	.292	-	.053	.060	.167	-	-	-
D	.033	.071	.450	.059	-	.097	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	.021	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	.567	.893	.550	.868	.436	.709	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-	.214	.076
G	.333	.036	-	.044	.287	.045	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.625	.924
H	.067	-	-	.015	.255	.149	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.161	-
Hk																						
n	52	23	64	61	31	23	39	27	16	19	41	40	26	14	25	16	36	24	24	26	56	20
A	-	-	-	-	.210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	.032	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	.258	-	-	-	-	-	-	-	-	-	-	-	-	-	.458	-	-	-
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.020	-	-	-	-	-	-	-
E	.952	.957	.930	.951	.371	.804	.795	.778	.813	.447	.854	.613	.846	.964	.680	1.000	.694	.208	.542	.885	-	.150
F	-	-	.016	-	.129	.196	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G	.038	.043	.055	.041	-	-	-	.037	-	-	-	.013	-	-	.040	-	-	-	-	-	.973	.850
H	.010	-	-	.008	-	-	.205	.185	.188	.553	.146	.375	.154	.036	.260	-	.306	.792	-	.115	.027	-
Mdh																						
n	12	40	22	15	14	22	27	21	21	19	18	19	16	10	13	25	24	24	13	24	29	30
A	1.000	1.000	1.000	.533	1.000	.932	1.000	.929	.548	.868	.639	.789	.750	.850	.962	1.000	.979	.917	1.000	1.000	.948	.833
B	-	-	-	.467	-	.068	-	.071	.452	.132	.361	.211	.250	.150	.038	-	.021	.083	-	-	.052	.167
He	0.322	0.258	0.452	0.337	0.544	0.455	0.149	0.353	0.364	0.418	0.385	0.474	0.392	0.372	0.457	0.316	0.314	0.297	0.448	0.250	0.282	0.255
Ho	0.371	0.314	0.424	0.431	0.575	0.472	0.197	0.335	0.461	0.407	0.445	0.441	0.444	0.384	0.478	0.284	0.374	0.366	0.443	0.299	0.280	0.290

differentiation was assessed according to the *F*-statistics (Wright 1931), and pairwise genetic distance values were calculated between all pairs of populations and averaged between groups of populations (Rogers 1972, Nei 1978). Dendrograms were constructed to show evolutionary relationships according to the UPGMA (Sneath & Sokal 1973) and the Wagner (Farris 1972) methods. Correlation tests between genetic and geographic distances used the Spearman coefficients in STATISTICA for Windows (ver. 5.1: StatSoft, USA, 1997). Levels of gene flow (Nm) were estimated from F_{ST} values using the equation: $F_{ST}=1/(4Nm+1)$ (Wright 1931).

Results

The glaciers divide the study populations into three coherent

Table III. Matrix of Nei's (1978) pairwise genetic distances (below the diagonal) and identities (above the diagonal) between the 22 populations studied. Numbers and abbreviations of populations as in Table I.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	INXP	-	0.913	0.925	0.906	0.881	0.917	0.889	0.741	0.653	0.634	0.714	0.645	0.721	0.723	0.751	0.789	0.729	0.574	0.708	0.701	0.648	0.597
2	ADCV	0.091	-	0.919	0.870	0.824	0.947	0.944	0.648	0.616	0.554	0.647	0.608	0.670	0.705	0.695	0.722	0.698	0.540	0.629	0.695	0.573	0.557
3	SPTV	0.078	0.084	-	0.862	0.833	0.934	0.820	0.718	0.633	0.578	0.682	0.650	0.714	0.739	0.750	0.773	0.714	0.533	0.719	0.733	0.499	0.477
4	NRTF	0.099	0.140	0.149	-	0.745	0.877	0.850	0.625	0.650	0.524	0.680	0.572	0.658	0.645	0.621	0.671	0.602	0.455	0.574	0.582	0.480	0.426
5	CPST	0.127	0.194	0.183	0.294	-	0.907	0.820	0.598	0.524	0.552	0.558	0.563	0.594	0.614	0.668	0.686	0.682	0.591	0.734	0.622	0.691	0.655
6	GODW	0.086	0.055	0.068	0.132	0.098	-	0.897	0.636	0.616	0.567	0.639	0.611	0.671	0.702	0.698	0.730	0.698	0.548	0.676	0.674	0.556	0.520
7	CPGL	0.117	0.058	0.198	0.162	0.199	0.109	-	0.588	0.557	0.550	0.579	0.535	0.587	0.599	0.614	0.666	0.683	0.603	0.573	0.583	0.651	0.598
8	CPWT	0.300	0.434	0.331	0.470	0.514	0.453	0.556	-	0.917	0.924	0.964	0.951	0.968	0.931	0.979	0.913	0.890	0.787	0.846	0.948	0.417	0.337
9	EPLT	0.426	0.485	0.458	0.431	0.647	0.485	0.585	0.087	-	0.913	0.997	0.962	0.995	0.965	0.919	0.920	0.918	0.816	0.823	0.927	0.381	0.360
10	EPNT	0.455	0.590	0.548	0.647	0.595	0.568	0.598	0.079	0.091	-	0.915	0.963	0.930	0.883	0.945	0.860	0.912	0.927	0.818	0.879	0.458	0.349
11	EPBC	0.337	0.435	0.382	0.385	0.583	0.449	0.546	0.036	0.003	0.089	-	0.959	1.000	0.962	0.947	0.931	0.910	0.792	0.832	0.939	0.399	0.362
12	EPNV	0.439	0.498	0.431	0.559	0.574	0.492	0.626	0.050	0.039	0.038	0.042	-	0.983	0.962	0.973	0.920	0.943	0.892	0.861	0.962	0.406	0.352
13	EPMC	0.327	0.401	0.336	0.419	0.521	0.399	0.533	0.032	0.005	0.073	0.000	0.017	-	0.993	0.976	0.963	0.946	0.820	0.883	0.976	0.402	0.373
14	EPSO	0.324	0.350	0.303	0.439	0.487	0.354	0.512	0.071	0.036	0.124	0.039	0.038	0.007	-	0.969	0.974	0.948	0.787	0.888	0.982	0.406	0.386
15	BRRK	0.286	0.364	0.287	0.476	0.403	0.359	0.487	0.021	0.084	0.057	0.055	0.028	0.025	0.032	-	0.938	0.945	0.854	0.899	0.964	0.471	0.411
16	MMGE	0.237	0.326	0.258	0.399	0.377	0.308	0.407	0.091	0.083	0.151	0.072	0.083	0.038	0.026	0.064	-	0.973	0.810	0.927	0.961	0.477	0.444
17	KAYI	0.316	0.359	0.336	0.508	0.383	0.360	0.382	0.117	0.085	0.093	0.094	0.059	0.055	0.054	0.056	0.028	-	0.926	0.914	0.951	0.528	0.499
18	TKGL	0.556	0.616	0.630	0.787	0.525	0.602	0.506	0.240	0.204	0.076	0.233	0.115	0.199	0.240	0.158	0.211	0.077	-	0.791	0.808	0.542	0.471
19	BUGL	0.345	0.464	0.330	0.555	0.309	0.392	0.558	0.167	0.195	0.200	0.183	0.150	0.124	0.119	0.106	0.076	0.090	0.235	-	0.881	0.456	0.424
20	APTI	0.356	0.364	0.311	0.541	0.475	0.394	0.539	0.054	0.076	0.129	0.063	0.039	0.025	0.018	0.036	0.040	0.051	0.213	0.127	-	0.404	0.393
21	CPKG	0.434	0.557	0.696	0.734	0.370	0.586	0.429	0.874	0.966	0.782	0.920	0.902	0.911	0.901	0.752	0.741	0.639	0.612	0.785	0.907	-	0.936
22	EMGI	0.515	0.586	0.741	0.853	0.423	0.653	0.515	1.088	1.015	1.054	1.017	1.043	0.985	0.953	0.889	0.813	0.696	0.752	0.859	0.934	0.066	-

groups of northern, central and southern, with the Aviator and Campbell glaciers forming north/central and central/south barriers, respectively. The northern group includes Emerging Island, Apostrophe Island and Cape King (20 to 22 in Fig. 1 and Table I), the central group contains 12 populations (8–19 in Fig. 1 and Table I), and the southern group includes the remaining seven populations (1–7 in Fig. 1 and Table I).

Allele frequencies observed at the five loci studied in the 22 populations (Table II) suggest four loci were highly polymorphic, with eight different alleles scored for *Pgm*, *Mpi* and *Hk*, and seven alleles for *Phi*. The locus *Mdh* was the least variable, with only two scored alleles. Some populations were remarkably polymorphic, such as Gondwana station (six alleles in the *Pgm* locus), Edmonson Point "Lake Top hill", Baker Rocks and Burns Glacier (seven alleles in the *Pgm* locus), and Kay Island (five alleles in the *Phi* locus).

Higher levels of within-population variability in *G. terranova* are also apparent from the rates of heterozygosity, averaged in each population for all five loci (Table II). In seven populations, the expected level of heterozygosity exceeds 40% (with the population of Cape Sastrugi having > 50%), and only in one population (Campbell Glacier) it is < 20%.

Table IV. Average genetic distance (Nei 1978) between populations of the same geographic group (in the diagonal) and between populations from different groups. Ranges for each comparison are given in parentheses.

	northern (+ Apostrophe Is.)	*central*	*southern*
northern	0.066		
central	0.877	0.090	
(+ Apostrophe Is.)	(0.612–1.088)	(0.000–0.240)	
southern	0.578	0.450	0.130
	(0.370–0.734)	(0.237–0.787)	(0.055–0.294)

No unique alleles were identified in the populations of *G. terranova* (Table II), though some allele combinations were peculiar to population groups. Furthermore, certain alleles exhibited remarkably different frequencies in different populations. For example, *Mpi-G* and *Hk-G* have high frequencies in the northern populations (Emerging Island and Cape King), *Mpi-F* is more frequent in the southern group populations, while *Mpi-A*, *Mpi-B*, *Mpi-C*, and *Pgm-G* were found unique to the 12 central group populations and Apostrophe Island. These data corroborate genetic differentiation congruently with the geographic position of populations. Furthermore, the Apostrophe Island population is more similar to the populations of the central group than to the geographically closer populations of Emerging Island and Cape King, an observation confirmed by genetic distance values calculated between all possible population pairs (Table III). In fact, the Apostrophe Island population has a lower average genetic distance (D = 0.073) from the central group populations compared with Emerging Island and Cape King (0.934 and 0.907, respectively). This places the Apostrophe Island population in the central group, and, furthermore, genetically closer to the southern group populations (average D = 0.426) than to the northern populations.

The average genetic distance between all populations is relatively low (D = 0.352), but distances exceeding D = 0.900 occur in comparisons involving Emerging Island and Cape King. The three described population groups, northern,

central (including Apostrophe Island) and southern, are genetically distinct, with average genetic distances within the same group never exceeding 0.130 (Table IV), while distances between populations from different groups range from 0.450 (central vs southern) to 0.877 (northern vs central).

With the sole exception of Apostrophe Island, genetic distances tend to correlate with geographic distances. The correlation is positive and highly significant (r = 0.5045; P = 0.0000) when all populations are considered, while removal of Apostrophe Island increases the correlation (r = 0.6679; P = 0.0000), as it does considering only the central group populations (r = 0.6667; P = 0.0000). A slightly lower (r = 0.4394), although still significant (P = 0.0463), correlation is observed among the southern group populations.

The populations of Edmonson Point identified as beach, microclimate station and "Lake Top hill" constitute a very uniform group, with low genetic distances (D < 0.005). These three collecting sites are very close to each other (< 500 m), but are still separated by areas of barren or ice-covered ground. The other three collecting sites have genetic distances comparable to those observed between different populations, as could be expected from the larger geographic distance separating them from the other collecting sites within Edmonson Point.

The analysis of the 22 populations via Wright's F-statistics confirms the three group hypothesis. Overall F_{ST} is 0.310, a value which decreases if the Emerging Island and Cape King populations are excluded (F_{ST} = 0.257). Even lower values of F_{ST} are found within the groups of central (F_{ST} = 0.122) and southern (F_{ST} = 0.155) populations. Estimates of gene flow (Nm), which can be obtained from F_{ST} values, are relatively low (< 1) if populations from different groups are considered, compared with estimates within populations from the same group (> 1).

The evolutionary dendrograms (Figs 2 & 3) have been constructed on the basis of genetic distance estimates and illustrate the relationships among the 22 populations. Both reconstructions show the populations arranged according to the three aforementioned geographic groups, though Apostrophe Island is included within the central populations group. The only difference between the two reconstructions is the location of the Cape Sastrugi population, which is associated with the southern group by UPGMA (Fig. 2) and with the northern populations in the Wagner tree (Fig. 3).

Discussion

This work represents the first study on genetic variability of any Antarctic soil arthropod. The small size of *G. terranova* prevented screening of more than five loci; these five appear to be among the most variable ones, possibly more variable than most of the loci which have not been used. Nevertheless, the electrophoretic data collected in this analysis can be directly compared with that obtained from temperate Collembola, enabling comparison of the rate of genetic variability in very different environmental conditions. The number of alleles per locus was higher compared to the same five loci in other European Collembola (Fig. 4), a difference which cannot be attributed to population number. Indeed, selecting a subset of populations (for example the seven populations of the southern group), the number of alleles scored remains high, or comparable with the high European scores (Fig. 4). A similar result is obtained by comparing the maximum number of different genotypes observed at each locus in a single population of *G. terranova* with that of other Collembola. The *Hk* locus, for example, yielded 12 different genotypes in the population of Cape Sastrugi, compared with a maximum of five in all other collembolan species indicated

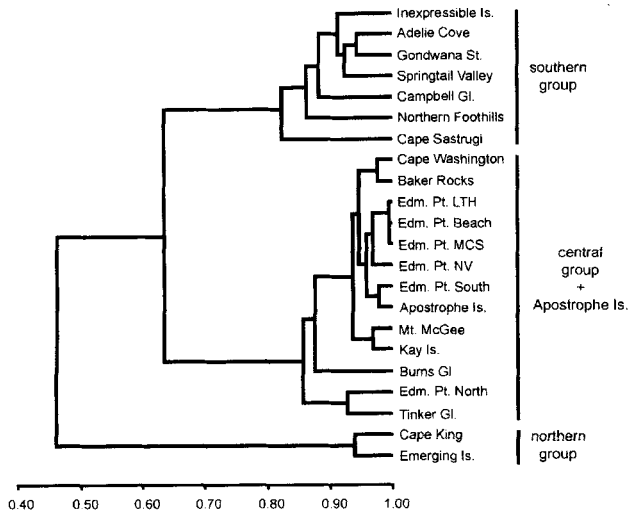

Fig. 2. UPGMA dendrogram of evolutionary relationships based on Nei's (1978) genetic distance. Population abbreviations as in Table I.

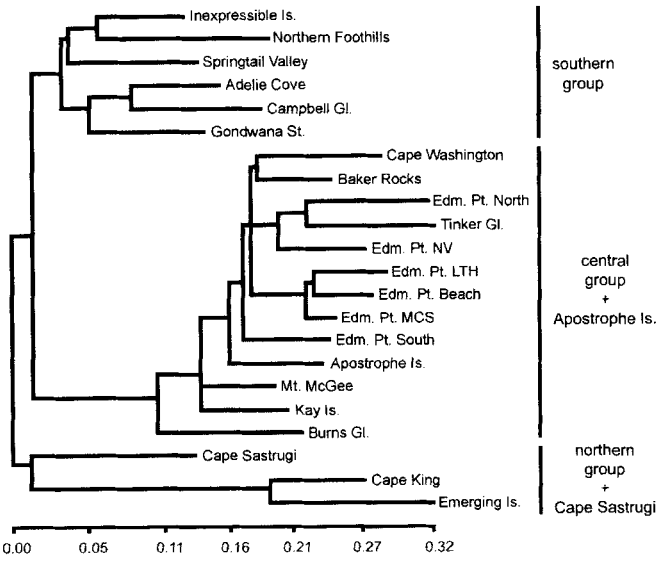

Fig. 3. Wagner tree depicting evolutionary relationships based on Rogers' (1972) genetic distance. Population abbreviations as in Table I.

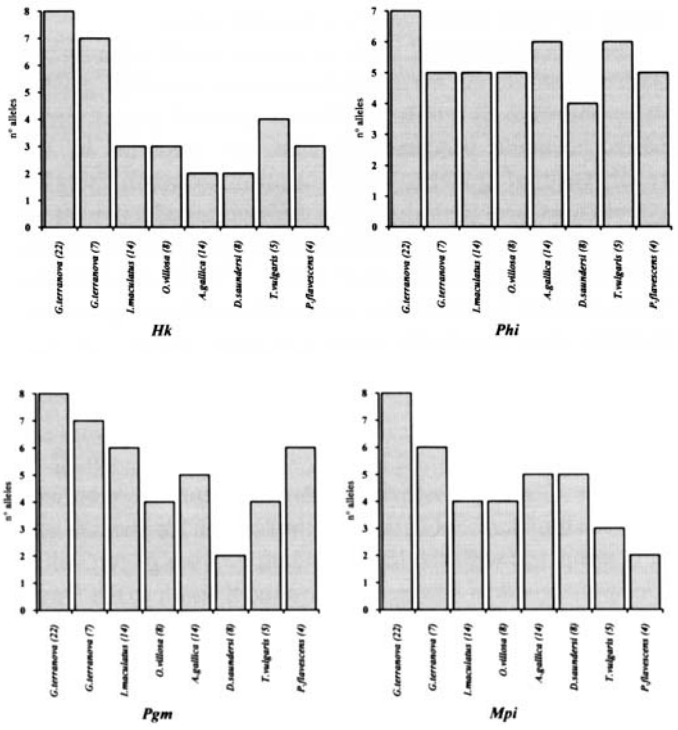

Fig. 4. Comparison of maximum numbers of alleles found in various species of Collembola for the four most polymorphic loci studied. The number of populations screened for each species is indicated within parentheses. Data for *G. terranova* refers to all 22 populations (first column) and to the seven populations of the southern group only (second column). Data for other species are from: Carapelli *et al.* (1997) (*Isotomurus maculatus* (Schäffer, 1896)); Frati *et al.* (1994) (*Orchesella villosa* (Geoffroy, 1762)); Fanciulli *et al.* (1994) (*Allacma gallica* (Carl, 1899)); Fanciulli *et al.* (1995) (*Dicyrtomina ornata* (Nicolet, 1842)); Fanciulli *et al.* (2000) (*Tomocerus vulgaris* (Tullberg, 1871) and *Pogonognathellus flavescens* (Tullberg, 1871)).

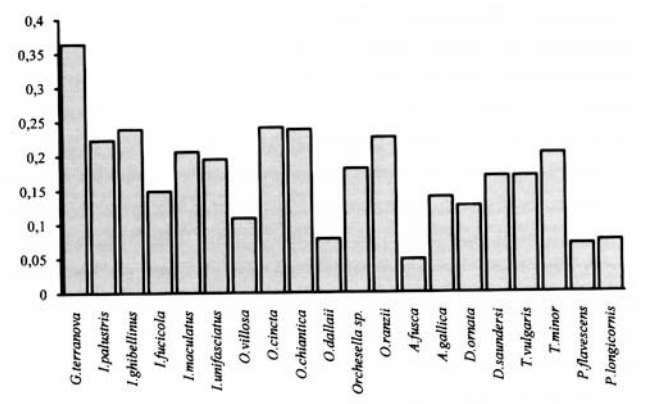

Fig. 5. Comparison of average heterozygosity estimates (He) of *G. terranova* with those obtained in other collembolan species. Data for other species are from: Carapelli *et al.* (1997) (*Isotomurus palustris* (Müller, 1776), *I. ghibellinus* (Carapelli et al., 1995), *I. fucicola* (Reuter, 1891), *I. maculatus*, *I. unifasciatus* (Schäffer, 1896)); Frati *et al.* (1994) (*Orchesella villosa*, *O. cincta* (Linné, 1758), *O. chiantica* (Frati and Szeptycki, 1990), *O. dallaii* (Frati and Szeptycki, 1990), *Orchesella* sp., *O. ranzii* (Parisi, 1960)); Fanciulli *et al.* (1994) (*Allacma fusca* (Linné, 1758), *A. gallica*); Fanciulli *et al.* (1995) (*Dicyrtomina ornata*, *D. saundersi* (Lubbock, 1862)); Fanciulli *et al.* (2000) (*Tomocerus vulgaris*, *T. minor* (Lubbock, 1862), *Pogonognathellus flavescens*, *P. longicornis* (Müller, 1776)).

in Fig. 4. The average heterozygosity for *G. terranova* is 36.4%, a much higher value than that observed for the same five loci in other collembolan species (Fig. 5). This comparison suggests *G. terranova* has a high level of genetic variability. Such high genetic variability, especially within populations, was not expected for a species living in a stable environment, where, indeed, a tendency towards genetic uniformity was expected (Nevo 1978, Nevo *et al.* 1994). These results suggest that, even if stable, the Antarctic environment may positively select high levels of polymorphism, as a survival strategy under extreme condition (*sensu* Hoffmann & Parsons 1997). While the Antarctic environment is ecologically simple and stable, it is very heterogeneous, and relatively unpredictable, on a small spatial and temporal scale, providing a possible explanation for the observed high levels of variability. An alternative explanation is the time since speciation or the last major bottleneck.

The genus *Gressittacantha* is endemic to Antarctica, and *G. terranova* may be quite an old Antarctic species, similar to what has been suggested for some Antarctic mites (Marshall & Pugh 1996, Marshall & Coetzee 2000, Pugh & Convey 2000), supporting the hypothesis that the species has not recently undergone remarkable population fluctuations (nor bottlenecks). Genetic variability may also depend upon population size (Kimura & Ohta 1971). No empirical estimates of populations size are currently available for *G. terranova*, though direct observations (up to 200–300 specimens concentrated under a single stone) and data collected for other collembolan species (Kennedy 1999), suggest that populations are quite large (several thousands of specimens), possibly contributing to preventing genetic drift from leading to allelic fixation and/or loss.

One final possibility is that higher variability is due to increased mutation rates, associated with high Antarctic levels of UV-B radiation (Kennedy 1995, Skotnicki *et al.* 2000). Comparing genetic variability with other Antarctic organisms suggests high levels of genetic variability (estimated via RAPD) in Victoria Land moss populations, for example *Bryum pseudotriquetum* (Hedw.) Gaertn., Meyer et Scherb. and *Hennediella heimii* (Hedw.) Zand. (Skotnicki *et al.* 1998a, Dale *et al.* 1999). This situation is also partially confirmed by data from the nematode *Scottnema lindsayae* Timm (Courtright *et al.* 2000), where multiple rDNA genotypes were found in the same population.

The current study suggests a low level of gene flow between populations, since these can be differentiated over very small

geographic distances. The Edmonson Point populations, excepting the three closely related collecting sites of beach, microclimate station and "Lake Top hill", show levels of genetic distance comparable with those observed between geographically isolated populations. Edmonson Point north is separated from the beach only by a narrow glacier tongue (< 100 m wide), and the site of Edmonson Point north valley is actually the northern part of the beach. The beach itself is not uniformly covered with mosses, and collembolan communities only occur in small areas where free snow-melt water allows patches of mosses to grow. Thus, even a well delimited site such as Edmonson Point may contain *G. terranova* populations which are effectively isolated one from another by areas of barren or ice-covered ground, a suggestion confirmed by the Edmonson Point north population clustering with the Tinker Glacier population, rather than with other Edmonson Point populations. We suggest that even small geographic barriers (narrow glaciers, areas of barren soil, etc.) can be effective mechanisms for genetic isolation. This conclusion is corroborated by data obtained from the mitochondrial COII gene in *Isotoma klovstadi* (Frati *et al.* 2000, in press), where geographically isolated populations share only a few or no common haplotype. The observed levels of gene flow also support this hypothesis, in that gene flow seems to be active only among populations of the same geographic group. Yet, when $Nm < 1$, as between populations from different geographic groups, gene flow is unable to counteract the effect of genetic drift (Wright 1931), leading to population genetic differentiation. Thus, the genetic structure of *G. terranova* appears to be strictly dependent on the geographic distance between its populations and on the local geographic barriers. Some restrictions to gene flow were detected also for the nematode *Scottnema lindsayae* (Courtright *et al.* 2000), and significant genetic divergence between geographically isolated populations was observed in populations of the mosses *Ceratodon purpureus* (Hedw.) Brid. (Skotnicki *et al.* 1998b) and *Hennediella heimii* (Dale *et al.* 1999) separated by 300 to 500 km. Antarctic mosses, however, may be dispersed by wind, which appears to be an effective homogenizing force (Skotnicki *et al.* 1998a, 2000, Dale *et al.* 1999).

The general correlation between geographic and genetic distances in populations of *G. terranova* suggests that geographic factors are most important in explaining population differentiation. In this context, a special role is played by the two major glaciers crossing the area studied: the Aviator and the Campbell. Our genetic data suggest that these glaciers constitute efficient barriers to the exchange of individuals between populations. The efficiency of geographic barriers is obviously related to the reduced vagility of these animals. Although Collembola are able to walk short distances even across snow (Lyford 1975, Hagvar 1995), there are no data for large distances. The Antarctic environment is made more complex by its spatial heterogeneity, where suitable habitats are separated by extremely hostile barriers, and even light winds can blow individuals to unsuitable areas.

The pattern observed in *G. terranova* could represent an intermediate step in the speciation process, in which genetic differentiation is arising between isolated populations. Microspeciation processes, in fact, are mediated by the insorgence of genetic differentiation between isolated populations, as a consequence of differential selective forces or, more often, as an effect of random genetic drift between isolated populations (Nei *et al.* 1983). If this is the case, the large glaciers, which appear to constitute efficient gene flow barriers, may eventually allow speciation events. In this context, it is also interesting to observe that the distribution of the main species of Collembola in Victoria Land is largely dependent upon glaciers (Wise 1967, Frati *et al.* 1997, Frati 1999). *Isotoma klovstadi* is the dominant species north of the Mariner Glacier, *Gressittacantha terranova* is dominant between the Mariner Glacier and the Nansen Ice Sheet, where it is sympatric with the less abundant *Friesea grisea*, while *Gomphiocephalus hodgsoni* is dominant south to the David Glacier. Interestingly, post-Gondwanan speciation, as a consequence of geographic isolation, has been suggested for the Antarctic mite genus *Maudheimia* (Marshall & Coetzee 2000).

The only significant exception to the correlation between geographic and genetic structuring of *G. terranova* populations is provided by the population of Apostrophe Island, which clearly belongs "genetically" to the central group. The most likely hypothesis to explain this pattern is that the population of Apostrophe Island represents a recent introduction of individuals from the area between the Aviator and the Campbell glaciers. This hypothesis is supported by the fact that the levels of genetic variability in this population are among the lowest of all populations studied (Table II), an observation consistent with the expected reduction of genetic variability due to the "founder effect" phenomenon (Mayr 1963). A slightly different scenario could be considered, implying the occurrence of waves of recolonization (the "rescue effect" *sensu* Brown & Kodrik-Brown 1977), which have been proposed as a model for insular biogeography, and have been suggested to explain the distribution of terrestrial arthropods in the South Sandwich Islands (Convey *et al.* 2000, Pugh & Convey 2000). This latter scenario, however, is only possible if passive transport of Collembola is a relatively frequent event. Passive transport over large distances may be mediated by winds and/or other animals, including man (Gressitt *et al.* 1960, Wise 1967), and, in other circumstances, passive transport has been claimed to explain the recent introduction of collembolan species such as *Protaphorura* sp., *Folsomia candida* (Willem), and *Cryptopygus caecus* (Wahlgren) in Eastern Antarctica (Greenslade & Wise 1984). Collembola have been caught in aerial traps in Antarctica (Gressitt *et al.* 1960). In fact, Antarctic winds are very dry, and soil arthropods are extremely sensitive to low humidity rates, suggesting wind to be a very unlikely factor for dispersal of Antarctic arthropods (Marshall & Coetzee 2000, Pugh & Convey 2000). On the

contrary, this appears to be an important factor for dispersal of mosses (Skotnicki *et al.* 1998a, 2000, Dale *et al.* 1999) or other invertebrates which possess resistant stages enhancing their dispersal capability. While ocean currents have been proposed for dispersal of Antarctic mites (Pugh & Convey 2000), another possible hypothesis implies the participation of birds. Apostrophe Island hosts a large community of skuas (*Catharacta maccormicki* Saunders) which are also abundant in many collecting sites of the central group, and which may be indicated as possible candidates for the transport of individual Collembola. These individuals could be entrapped in small pieces of soil or stones attached to their feet. The same phenomena of local immigration and transport may also be claimed to explain the enigmatic position of the population of Cape Sastrugi, which is placed with the northern populations in the Wagner tree (Fig. 3).

This work has suggested the influence of the Antarctic environment on the genetic differentiation of geographically isolated populations of *G. terranova*. This data are concordant with those obtained for another collembolan species from Victoria Land, *Isotoma klovstadi*, where noticeable genetic differentiation between isolated populations was found using mitochondrial DNA sequences (Frati *et al.* 2000, in press). Future work will be needed to confirm the influence of the Antarctic environment on the structuring of other collembolan species (for example *Gomphiocephalus hodgsoni*, *Friesea grisea*), and to test the consistency of such results using different genetic markers, including allozymes and DNA sequences. In this context, it will also be of great help to perform similar studies on species from the Antarctic Peninsula, with particular reference to *Friesea grisea*, which is the only know collembolan species to occur in both Eastern and Western Antarctica (Greenslade 1995).

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