Geographic scaling and genetic differentiation in two highly mobile European saltmarsh beetles

Konjev Desender and Peter Verdyck

Royal Belgian Institute of Natural Sciences, Department of Entomology
Vautierstraat 29, B-1000 Brussels (Belgium)

ABSTRACT. Genetic structure and diversity are studied in two European saltmarsh beetles, *Bembidion minimum* and *B. normannum*, on a regional as well as a Western European scale. Results are based on allozymes, studied at four polymorphic loci for more than 1600 individuals from all remaining saltmarshes in Belgium and from a selection of European reference sites. Average gene diversity is not related to habitat or population size, but is larger in the more common *B. minimum*, in comparison to Atlantic samples of *B. normannum*. One Mediterranean sample of the latter species reveals a much higher diversity and suggests this region as the evolutionary centre of origin and/or as a possible glacial refugium of the species. Significant overall genetic structure is observed in the complete data of both species, with 2 to 6 % of the total genetic variation explained by differentiation between populations. Genetic differentiation in both species is significant at different geographic scales, with higher values at a larger scale. A Mantel-test (isolation by distance) between geographic and genetic distance is significant in *B. normannum*. Our results indicate that habitat fragmentation has not yet resulted in genetic erosion, probably because of the large population sizes of both species, even in very small saltmarshes. The observed genetic differentiation suggests that metapopulations at a relatively large geographic scale are still functional in these highly mobile species. Re-establishment of even small saltmarshes is suggested as a positive conservation measure for long term survival of these specialised ground beetles.

KEY WORDS : Carabidae, European saltmarshes, *Bembidion minimum*, *Bembidion normannum*, population genetics, habitat fragmentation, genetic diversity, metapopulation structure, dispersal power, geographic scale.

INTRODUCTION

Insects prove useful models and indicators of geographic structure and genetic differentiation in relation to habitat fragmentation and isolation. Nowadays, populations of many terrestrial arthropods only survive in remnants of natural habitats, highly isolated from each other. This is certainly the case for many habitat types in Western Europe and in particular within the region of Flanders (Belgium). Fragmentation in general results in a reduced genetic diversity (e.g. ANDREN, 1994; AVISE & HAMRICK, 1996; FRANKHAM, 1996), but may also increase genetic differentiation between populations as a result of reduced gene flow (SLATKIN, 1994).

Population genetics studies the distribution and abundance of genotypes between and within natural populations. Geographic genetic structure and genetic diversity combine both demographic and genetic processes, such as extinction/recolonisation and metapopulation dynamics, gene flow, genetic drift and natural selection. Ground beetles (Coleoptera, Carabidae) appear to be ideal model organisms for such studies. In Western Europe, carabids belong to the most popular, diversified and best-studied invertebrates. They show a stable taxonomy and a pronounced habitat preference, and their large- and small-scale distribution is relatively well known. Abundant data on their occurrence are available for the last 150 years, especially in the Netherlands and Belgium (TURIN, 2000). The small size of most ground beetles, as well as their extreme diversity and high abundances, enable investigations on the effects of geographical scaling on genetic structure and diversity, while population genetic data can be relevant for conservation ecological purposes. Ground beetles also show wide variation in traits related to dispersal power. More importantly, potential gene flow can be directly or indirectly quantified by studying the morphology of the hind wings (macroptery, brachyptery or polymorphism) and flight muscles. Based on such data, model species with known but varying dispersal power

and mobility can be compared in population genetic studies. Expected gene flow, as deduced from knowledge on dispersal power and commonness/rarity, can be confronted with gene flow estimates derived from genetic studies. Many carabid species even show a dispersal di- or polymorphism and such wing polymorphic species have enabled test and confirmation of the dispersal-gene flow hypothesis ('levels of gene flow among populations are correlated with dispersal power') (DESENDER & SERRANO, 1999; PETERSEN & DENNO, 1997).

In Western Europe and especially in Flanders, natural habitats have severely suffered from human impact and have decreased in size, number and quality. Therefore these habitats have become more and more fragmented. Habitats that have, in recent historical times, decreased dramatically in size are forests and saltmarshes (TACK et al., 1993; DIJKEMA et al., 1984). In a recently published 'Red list' of the ground beetles in Flanders the conclusion was reached that many typical saltmarsh species have become either rare, endangered and close to extinction, or probably even became extinct during recent decades (DESENDER et al. 1995). Similar patterns have been observed in other European countries (DESENDER & TURIN 1989). The main reasons for this general decrease are most probably habitat destruction and reduction, as well as pollution, which is known to be severe in estuaries and coastal ecosystems (DIJKEMA et al. 1984; WESTHOFF, 1985).

To study the impact of fragmentation, a regional, inter-institutional and conservation genetic study project was conducted in Flanders (for more details, see DESENDER et al., 1998). Within this project, case studies were performed on a large array of organisms, including many invertebrates (DE MEESTER et al., 2000). Insects were studied from either forest fragments (specialised forest dwelling beetles, cf. DESENDER et al., 1999) or isolated saltmarshes (halobiontic or halophilic ground beetles, cf. DESENDER et al., 1998).

In the present study, we investigate the genetic structure in two highly mobile saltmarsh beetle species of the genus *Bembidion*, *B. minimum* and *B. normannum*, on a regional as well as western European scale. We test whether effects of fragmentation are visible in species with high mobility, where we expect little or no differentiation. Results are based on allozyme electrophoresis, investigated by studying samples from all remaining saltmarshes in Belgium and some European reference sites at larger distances. Neither species has previously been studied with respect to its population genetics. They persist in at least some of the Flemish saltmarshes. In particular, we are interested in the effect of different geographic scaling on the observed genetic differentiation and diversity, with implications for metapopulation size estimation and conservation genetics. We will therefore relate the within-population genetic diversity and among-population genetic differentiation in both species

to habitat size, population size and geographic distance between habitats.

MATERIAL AND METHODS

Study species

Bembidion (Emphanes) minimum Fabricius, 1792 is a small (total length about 2.7 mm), metallic-black halophilic ground beetle with a Palearctic distribution (Fig. 1 left). Its range extends from southern Scandinavia to southern Europe and eastwards as far as Siberia. The species is typically found in high densities (sometimes up to some 20 ind./m², cf. DESENDER & SEGERS, 1985 and DESENDER, unpublished data) in saltmarshes on marine clay soils, mainly in coastal estuaries. There are also inland observations from saline or brackish areas or from polder areas (DESENDER & MAELFAIT, 1999; TURIN, 2000). The species is active during daytime and reproduces in spring. *B. minimum* is constantly macropterous and, as far as studied, shows functional flight musculature. There are numerous flight observations (DESENDER, 1989) and the species is an excellent swimmer/floater (TURIN, 2000). In Flanders, it has recently been classified as being still at relatively low risk (not yet endangered) (DESENDER et al., 1995).
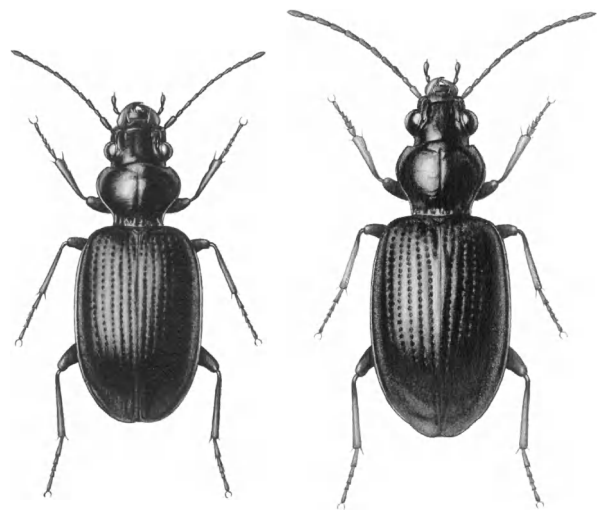

Fig. 1. – *Bembidion minimum* (left) and *B. normannum* (right): two sibling ground beetle species from saltmarshes, respectively about 2.7 and 3 mm (total length).

Bembidion normannum Dejean, 1831 is a somewhat larger (about 3 mm; Fig. 1 right), halobiontic sibling species of *B. minimum*, morphologically differentiated by its lighter leg colour, slightly different shape of pronotum and a more pronounced lighter apical spot on the elytra. This small carabid only occurs along the European coast, from southern Denmark to Italy. It is a typical inhabitant of marine saltmarshes and is also known from some inland high salinity sites (always in the vicinity of the coast). Although population densities can be locally quite high, *B. normannum* is much rarer than *B. minimum*.

Today, it survives in Flanders in a very restricted number of saltmarshes (see later). It has been categorised as 'vulnerable' in the Red data-book for Flanders (DESENDER et al., 1995). *B. normannum* is also active during daytime, reproduces during spring, is constantly macropterous and, as far as studied, always shows functional flight musculature (DESENDER, 1989; DESENDER & MAELFAIT, 1999).

Study sites and sampling

Saltmarsh ground beetles have been collected between 1992 and 1998 for genetic (electrophoretic) studies at different levels of spatial scale in populations with varying size and isolation. Fig. 2 shows the 24 sample locations of the two *Bembidion* species. Samples were taken in all remaining saltmarshes in Belgium and the adjacent southern part of the Netherlands (estuary of the river Schelde) (Fig. 2, sites 1-9) and from European reference sites at larger distances. In the Netherlands, population samples were taken from a saltmarsh area in Friesland (site 10). Other samples were collected in the UK: Morecambe Bay
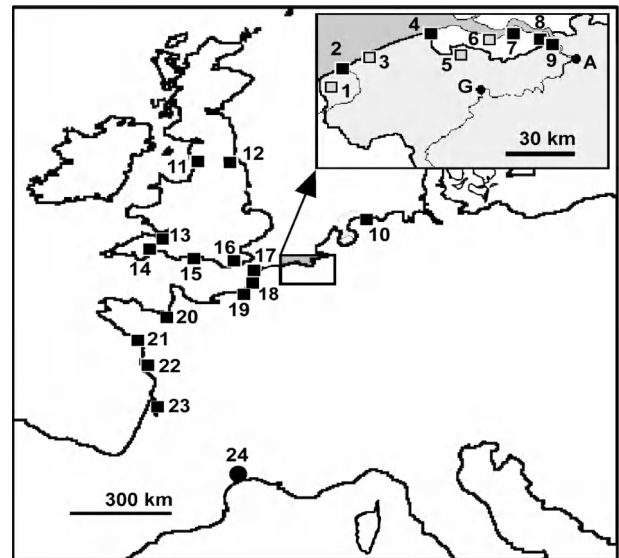

Fig. 2. – Location of the studied saltmarsh areas in Western Europe and in the region of Flanders and the southern part of the Netherlands (inset) (see Table 1 for details).

TABLE 1

Sampled saltmarshes (with information on habitat size and age) and populations of *Bembidion minimum* and *B. normannum* (with estimated total population sizes)

map code (cf. Fig. 2)	site/estuary	population code	salt-marsh area (ha)	salt-marsh age (y)	B. minimum	B. minimum number code	B. minimum population size	B. normannum	B. normannum number code	B. normannum population size
1	De Moeren	MOE	0.5	380	MOE	9	2000			
2	IJzer estuary	NIE	16	900	NIE	11	98133			
3	Oostende	OOS	0.1	250	OOS	12	1100			
4	Zwin	ZWC	120	700	ZWC	17	177143	ZWC	16	485714
"	"	ZWR	5	50	ZWR	18	50000	ZWR	17	485714
5	Molenkreek	MOL	0.5	300				MOL	10	1150
6	Braakman	BRA	1.5	300				BRA	2	1583
7	Ossenisse	OSS	50	700	OSS	13	200000			
8	Saeftinghe	SAE	300	700	SAE	14	251351			
9	Doel	DOE	51	80	DOE	3	53833			
10	Friesland	FER	400	200	FER	5	766667			
"	"	HOL	300	200	HOL	6	3133333	HOL	8	4433333
"	"	HOR	200	200	HOR	7	1300000			
11	Morecambe Bay	MOR	80	800	MOR	10	650667			
12	Humber estuary	HUM	200	?	HUM	8	1458333	HUM	9	1366667
13	Severn estuary	SEV	100	1000	SEV	15	350000			
14	Exe estuary	EXE	70	800	EXE	4	136111			
15	Thorney Island	THO	2	200				THO	15	3667
16	Rye estuary	RYE	10	700				RYE	14	50667
17	Authie estuary	AUT	250	400	AUT	1	1000000	AUT	1	375000
18	Canche estuary	CAN	200	1000	CAN	2	220000	CAN	3	380000
19	Somme estuary	SOM	200	2500	SOM	16	1966667			
20	Mont St Michel	MSB	500	7000				MSB	11	2500000
"	"	MSG	500	7000				MSG	12	1200000
21	la Guérande	GUA	2	400				GUA	6	4200000
"	"	GUB	20	1150				GUB	7	25400000
22	la Gachère	GAC	80	3500				GAC	4	313333
23	Gironde estuary	GIR	5	8000				GIR	5	13167
24	Rousillon	ROU	100	?				ROU	13	458333

(site 11), the Humber estuary (site 12), the Severn estuary (site 13), the Exe estuary (site 14), Thorney Island (site 15) and the Rye estuary (site 16). In France, samples were taken from the Bay of the Authie, Canche and Somme (sites 17-19), at the Mont St Michel (site 20), la Guérande (site 21 near the Loire estuary), La Gachère (site 22) and the Gironde estuary (site 23). Finally, for *B. normannum*, a sample from a Mediterranean saltmarsh at Bages in the Roussillon (France; site 24) was also studied.

Beetles were gathered by standardised hand collecting (per unit of time effort), transported alive to the lab, counted and identified under a binocular microscope. Subsequently, they were killed and stored in liquid nitrogen until electrophoresis. At some of the sampling sites absolute abundance estimates were made by means of a combined quadrat-flotation technique (DESENDER & SEGERS, 1985) in order to calibrate handcatches to densities.

Table 1 summarises information on the study sites, refers to their locations (as illustrated in Fig. 2), mentions population codes, as well as estimates of saltmarsh size (area) and age (when available). Number codes are also given for all populations studied for each species along with estimates of total population sizes. Such estimates are approximate because of the difficulty of estimating which areas of larger saltmarshes are actually inhabited by a given species. Nevertheless, we considered it better to incorporate a rough estimate of population density than to take only the area of a site into account. The age of saltmarshes was estimated using historical information, and is partly drawn from DESENDER (1985), GOETGHEBEUR (1976), HOFFMANN (1986) and HOUTHUYS et al. (1993). These estimates are maximum values of the historically documented existence of a particular site or area and are therefore less reliable for older sites. Varying ages or levels of spatial scale are included in our sampling design.

Allozyme electrophoresis

Samples were prepared for electrophoresis by homogenising the body (one elytrum was kept as morphological reference material) of individual beetles in 30 μl of distilled water on ice. Cellulose acetate electrophoresis (HEBERT & BEATON, 1989) permitted the examination of each individual for allelic variation. After a pilot study with 26 different enzymes, four polymorphic loci were selected in both *B. minimum* and *B. normannum*. These loci were chosen because they could be easily interpreted and scored, and because they were highly polymorphic (95%-criterion). For each gel, at least one reference individual was included for comparison. Continuous electrophoresis was carried out using standard methods (HEBERT & BEATON 1989. Two buffer systems were used: Tris-Glycine 10% (pH 8.5; HEBERT & BEATON 1989) and Tris-Maleate (pH 7.8; RICHARDSON et al. 1986). Samples from 18 and 17 populations were analysed for each species respectively (Table 1), yielding information on more than

800 individuals per species (at least 20 to 50 individuals from each population, if available).

The enzyme loci studied for *B. minimum* were Peptidase-D (dipeptide substrate: Phenylalanine Proline, PEPD, E.C. 3.4.-.-) and Mannose Phosphate Isomerase (MPI, E.C. 5.3.1.8) on a Tris-Maleate buffer and Glucose-6-phosphate Isomerase (GPI, E.C. 5.3.1.9) and Aldehyde Oxidase (AO, E.C. 1.2.3.1) on a Tris-Glycine buffer. Enzymes studied for *B. normannum* were AO and MPI on a Tris-Maleate buffer and GPI and Phosphoglucomutase (PGM, E.C. 2.7.5.1) on Tris-Glycine. We used slightly modified staining protocols from the ones outlined in HEBERT & BEATON (1989). In agreement with most studies on other animals (HEBERT & BEATON, 1989) and insects in particular (WARD et al. 1992), AO, GPI and PEPD showed a dimeric quaternary structure, while MPI and PGM were monomeric.

Analysis of enzyme allelic frequencies

Basic analyses were performed using BIOSYS-1 (SWOFFORD & SELANDER, 1981) and GENEPOP (v. 3; update from v. 1.2: RAYMOND & ROUSSET 1995a). Analyses were run with the four polymorphic enzymes (95%-criterion) in both species. Genetic diversity estimates were based on all scored loci. Allele frequency tables and basic genetic variability measures were produced with POP100GENE v1.03 (PIRY & BOUGET, 1999).

Genotype frequencies were first tested against Hardy-Weinberg expectations using an exact test procedure (ROUSSET & RAYMOND, 1995) and showed four significant deviations out of 72 tests for *B. minimum* and no significant deviations out of 56 tests for *B. normannum*. These results can be expected by chance alone and suggest that studied populations were all in Hardy-Weinberg equilibrium. The independence of the different markers used was investigated as described by RAYMOND & ROUSSET (1995b) in an exact probability test for genotypic linkage disequilibrium between each pair of loci for each population. Not a single significant linkage test-value was obtained in 108 tests for *B. minimum* and 70 tests for *B. normannum* (Bonferroni-corrected p-values). We can therefore safely conclude that no linkage was observed across all populations.

GENETIX v3.3 (BELKHIR et al., 1996-1998) was used to obtain, at different spatial scales, a variety of genetic differentiation estimates most widely used in recent population genetic studies. These included: F_{ST} (WEIR & COCKERHAM, 1984), G_{ST} (NEI, 1977) and G_{ST} -unbiased estimate (Nei & CHESSER, 1983). GENETIX also enables testing the significance of the F_{ST} -estimate (WEIR & COCKERHAM,1984), by means of a permutation procedure (estimate of the probability value of departure from the null hypothesis).

Within each species, genetic differentiation was tested between all pairs of populations with adjusted probability levels to avoid errors from multiple testing (sequential

Bonferroni method; RICE, 1989). Genetic distances between populations were visualised in dendrograms: Rogers' genetic distance (ROGERS, 1972) and Nei's unbiased genetic distance (NEI, 1978) were used to construct UPGMA-dendrograms for the different populations in both species. Bootstrap-values (1000 replicates) were estimated for each node of the dendrograms by means of TFPGA v1.3 (MILLER, 1997).

Isolation by distance was tested statistically by determining the significance of the correlation between (1) a matrix of Rogers' genetic distance estimates and (2) a matrix of Euclidian geographical distances. To this end, a Mantel-test was performed with p-values determined by a permutation procedure (as implemented in TFPGA and GENEPOP). The results of such a Mantel-test enable examination of the relative importance of gene flow as compared to drift and selection, and inspection for eventual geographical patterns in the data.

RESULTS

General population genetic analyses

Allele frequencies of the polymorphic loci, genetic variability measures and sample sizes are given for each population in Table 2 (*B. minimum*) and Table 3 (*B. nor-*

TABLE 2

Allele frequency table for four polymorphic enzymes studied in *Bembidion minimum*, along with calculated genetic variability estimates and sample sizes

LOCUS/POPULATION		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	TOTAL
Number of beetles (n)		36	22	19	33	22	43	22	53	30	56	138	93	44	30	47	59	43	14	804
A0	Allele																			Means
Gene Number (2n)		72	44	38	66	44	86	44	106	60	110	272	186	86	60	94	118	80	28	
Allele Number		3	4	2	2	2	2	2	2	3	2	3	3	4	2	2	4	3	2	2.611
	1	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.017	0.000	0.000	
	2	0.472	0.386	0.789	0.803	0.750	0.581	0.659	0.792	0.633	0.927	0.732	0.645	0.733	0.833	0.670	0.458	0.800	0.786	
	3	0.500	0.568	0.211	0.197	0.250	0.419	0.341	0.208	0.333	0.073	0.261	0.339	0.244	0.167	0.330	0.508	0.163	0.214	
	4	0.028	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.007	0.016	0.012	0.000	0.000	0.017	0.037	0.000	
Heterozygote Proportion (Hobs)		0.583	0.545	0.316	0.394	0.318	0.419	0.318	0.264	0.600	0.109	0.368	0.452	0.395	0.333	0.489	0.508	0.400	0.429	0.402
Gene Diversity (Hexp)		0.534	0.539	0.341	0.321	0.384	0.492	0.460	0.332	0.495	0.136	0.398	0.471	0.408	0.282	0.447	0.536	0.336	0.349	0.403
MPI	Allele																			Means
Gene Number		72	44	38	66	44	86	44	102	60	74	184	170	88	54	88	116	78	26	
Allele Number		2	2	3	2	2	2	2	2	2	2	3	2	2	2	3	2	2	2	2.167
	1	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.023	0.000	0.000	0.000	
	2	0.292	0.432	0.263	0.318	0.159	0.256	0.318	0.382	0.183	0.432	0.174	0.241	0.136	0.444	0.261	0.250	0.231	0.154	
	3	0.708	0.568	0.684	0.682	0.841	0.744	0.682	0.618	0.817	0.568	0.821	0.759	0.864	0.556	0.716	0.750	0.769	0.846	
Heterozygote Proportion		0.361	0.409	0.579	0.455	0.318	0.372	0.364	0.608	0.367	0.378	0.217	0.271	0.273	0.444	0.455	0.397	0.256	0.154	0.371
Gene Diversity		0.419	0.502	0.472	0.441	0.274	0.385	0.444	0.477	0.305	0.498	0.298	0.368	0.238	0.503	0.423	0.378	0.360	0.271	0.392
PEPD	Allele																			Means
Gene Number		72	44	38	66	44	82	44	106	60	112	274	186	88	60	94	118	84	28	
Allele Number		2	2	2	3	2	2	2	2	2	3	3	2	2	2	2	4	2	2	2.278
	1	0.181	0.045	0.079	0.091	0.205	0.037	0.023	0.057	0.100	0.071	0.084	0.054	0.068	0.100	0.064	0.186	0.060	0.179	
	2	0.819	0.955	0.921	0.894	0.795	0.963	0.977	0.943	0.900	0.911	0.909	0.946	0.932	0.900	0.936	0.797	0.940	0.821	
	3	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.018	0.007	0.000	0.000	0.000	0.000	0.008	0.000	0.000	
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	
Heterozygote Proportion		0.083	0.000	0.053	0.091	0.045	0.073	0.045	0.075	0.200	0.143	0.080	0.043	0.045	0.133	0.043	0.136	0.024	0.071	0.077
Gene Diversity		0.300	0.089	0.149	0.195	0.333	0.071	0.045	0.108	0.183	0.167	0.168	0.102	0.129	0.183	0.121	0.333	0.113	0.304	0.172
PGI	Allele																			Means
Gene Number		72	44	38	64	44	84	44	98	60	76	258	170	86	60	90	114	70	18	
Allele Number		4	3	3	3	3	4	3	3	3	3	3	3	4	4	3	4	3	3	3.278
	1	0.014	0.045	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	
	2	0.056	0.000	0.263	0.125	0.091	0.131	0.068	0.122	0.150	0.158	0.159	0.182	0.128	0.167	0.144	0.044	0.100	0.056	
	3	0.833	0.773	0.632	0.734	0.818	0.69	0.864	0.847	0.800	0.829	0.748	0.782	0.733	0.733	0.722	0.807	0.814	0.889	
	4	0.097	0.182	0.105	0.141	0.091	0.167	0.068	0.031	0.050	0.013	0.093	0.035	0.128	0.083	0.133	0.123	0.086	0.056	
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.026	0.000	0.000	
Heterozygote Proportion		0.333	0.455	0.474	0.469	0.364	0.524	0.273	0.224	0.267	0.237	0.411	0.329	0.372	0.433	0.311	0.316	0.200	0.222	0.345
Gene Diversity		0.297	0.376	0.535	0.432	0.321	0.484	0.251	0.270	0.341	0.292	0.408	0.356	0.436	0.434	0.445	0.334	0.324	0.216	0.364
ALL LOCI																				
Mean Allele Number		2.750	2.750	2.500	2.500	2.250	2.500	2.250	2.250	2.500	2.500	3.000	2.500	3.000	2.500	2.500	3.500	2.500	2.250	
Standard deviation		0.957	0.957	0.577	0.577	0.500	1.000	0.500	0.500	0.577	0.577	0.000	0.577	1.155	1.000	0.577	1.000	0.577	0.500	
Mean Heterozygote proportion		0.340	0.352	0.355	0.352	0.261	0.347	0.250	0.293	0.358	0.217	0.269	0.274	0.271	0.336	0.324	0.339	0.220	0.219	
Standard deviation		0.205	0.242	0.229	0.177	0.146	0.193	0.141	0.225	0.175	0.121	0.151	0.171	0.160	0.144	0.203	0.157	0.156	0.153	
Mean Gene Diversity		0.387	0.377	0.374	0.347	0.328	0.358	0.300	0.297	0.331	0.273	0.318	0.324	0.303	0.351	0.359	0.395	0.283	0.285	
Standard deviation		0.113	0.204	0.170	0.115	0.045	0.197	0.195	0.153	0.129	0.164	0.112	0.157	0.145	0.145	0.159	0.096	0.114	0.056	

TABLE 3

Allele frequency table for four polymorphic enzymes studied in *Bembidion normannum* along with calculated genetic variability estimates and sample sizes

LOCUS/POPULATION	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TOTAL
Number of beetles (n)		45	18	38	46	63	41	50	75	35	62	46	44	45	55	43	58	57	821
AO	**Allele**																		**Means**
Gene Number (2n)		84	22	74	90	124	78	94	148	68	122	92	88	84	110	82	116	114	
Allele Number		3	2	3	3	3	3	3	3	2	3	2	2	3	3	3	2	2	2.647
	1	0.048	0.045	0.041	0.011	0.008	0.038	0.032	0.027	0.015	0.016	0.000	0.034	0.250	0.045	0.159	0.043	0.035	
	2	0.929	0.955	0.946	0.944	0.968	0.949	0.957	0.966	0.985	0.975	0.978	0.966	0.595	0.945	0.817	0.957	0.965	
	3	0.024	0.000	0.014	0.044	0.024	0.013	0.011	0.007	0.000	0.008	0.022	0.000	0.155	0.009	0.024	0.000	0.000	
Heterozygote Proportion (Hobs)		0.095	0.091	0.108	0.089	0.065	0.103	0.085	0.068	0.029	0.049	0.043	0.068	0.500	0.073	0.220	0.052	0.070	0.106
Gene Diversity (Hexp)		0.137	0.091	0.105	0.107	0.063	0.100	0.083	0.066	0.029	0.049	0.043	0.067	0.566	0.105	0.310	0.083	0.068	0.122
MPI	**Allele**																		**Means**
Gene Number		90	32	76	4	74	76	4	62	44	44	90	84	90	22	84	34	52	
Allele Number		2	2	2	1	1	1	1	2	1	1	2	3	4	2	2	1	1	1.706
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.200	0.091	0.000	0.000	0.000	
	3	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.024	0.133	0.000	0.024	0.000	0.000	
	4	0.989	0.969	0.987	1.000	1.000	1.000	1.000	0.984	1.000	1.000	0.978	0.964	0.656	0.909	0.976	1.000	1.000	
	5	0.011	0.000	0.013	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Heterozygote Proportion		0.022	0.063	0.026	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.044	0.071	0.511	0.182	0.000	0.000	0.000	0.056
Gene Diversity		0.022	0.063	0.026	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.044	0.070	0.518	0.173	0.047	0.000	0.000	0.059
PGI	**Allele**																		**Means**
Gene Number		82	32	76	90	120	78	96	150	60	122	92	88	90	106	84	114	106	
Allele Number		3	3	3	2	2	3	2	2	3	2	2	2	2	3	2	2	2	2.353
	1	0.159	0.063	0.197	0.156	0.108	0.090	0.063	0.160	0.117	0.074	0.043	0.045	0.011	0.151	0.095	0.140	0.198	
	2	0.817	0.906	0.789	0.844	0.892	0.897	0.938	0.840	0.867	0.926	0.957	0.955	0.989	0.840	0.905	0.860	0.802	
	3	0.024	0.031	0.013	0.000	0.000	0.013	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	
Heterozygote Proportion		0.317	0.188	0.316	0.267	0.217	0.205	0.125	0.267	0.267	0.148	0.087	0.091	0.022	0.245	0.190	0.281	0.321	0.209
Gene Diversity		0.310	0.179	0.342	0.266	0.195	0.189	0.118	0.271	0.239	0.138	0.084	0.088	0.022	0.275	0.174	0.243	0.321	0.203
PGM	**Allele**																		**Means**
Gene Number		62	32	72	84	118	68	88	138	68	124	90	76	78	96	52	110	102	
Allele Number		2	2	2	2	3	2	3	2	2	3	3	2	5	2	2	3	3	2.529
	1	0.081	0.156	0.181	0.143	0.076	0.029	0.102	0.290	0.044	0.210	0.144	0.132	0.013	0.125	0.135	0.118	0.118	
	2	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.013	0.000	0.000	0.000	0.010	
	3	0.919	0.844	0.819	0.857	0.907	0.971	0.886	0.710	0.956	0.766	0.844	0.868	0.833	0.875	0.865	0.864	0.873	
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.011	0.000	0.128	0.000	0.000	0.018	0.000	
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	
Heterozygote Proportion		0.097	0.188	0.306	0.238	0.169	0.059	0.227	0.348	0.088	0.306	0.267	0.211	0.231	0.208	0.192	0.236	0.216	0.211
Gene Diversity		0.151	0.272	0.300	0.248	0.173	0.058	0.206	0.415	0.086	0.371	0.269	0.232	0.292	0.221	0.238	0.242	0.227	0.235
ALL LOCI																			
Mean Allele Number		2.500	2.250	2.500	2.000	2.250	2.250	2.250	2.250	2.000	2.250	2.250	2.250	3.500	2.500	2.250	2.000	2.000	
Standard deviation		0.577	0.500	0.577	0.816	0.957	0.957	0.957	0.500	0.816	0.957	0.500	0.500	1.291	0.577	0.500	0.816	0.816	
Mean Heterozygote proportion		0.133	0.132	0.189	0.148	0.113	0.092	0.109	0.179	0.096	0.126	0.110	0.110	0.316	0.177	0.151	0.142	0.152	
Standard deviation		0.128	0.065	0.145	0.126	0.098	0.087	0.094	0.153	0.119	0.135	0.106	0.068	0.235	0.074	0.101	0.137	0.144	
Mean Gene Diversity		0.155	0.151	0.193	0.155	0.108	0.087	0.102	0.196	0.088	0.139	0.110	0.114	0.350	0.193	0.192	0.142	0.154	
Standard deviation		0.119	0.095	0.152	0.125	0.092	0.079	0.085	0.180	0.106	0.165	0.108	0.079	0.249	0.072	0.112	0.121	0.146	

mannum). In general, *B. minimum* shows a distinctly larger genetic variation within populations than does *B. normannum* (based on all calculated genetic diversity measures; Table 2). Mean gene diversity ranges between 0.273 and 0.395 for *B. minimum* and only between 0.087 and 0.193 for *B. normannum* (Atlantic samples). The Mediterranean sample of the last-mentioned species in its turn shows a much higher genetic diversity ($H_{exp} = 0.350$) than Atlantic samples (Table 3).

F-statistics are summarised for both species in Table 4. Results of significant pairwise exact tests for genetic differentiation between populations are given in Table 5 (*B. minimum*) and Table 6 (*B. normannum*).

Overall genetic differentiation is highly significant for both species and for each polymorphic enzyme (Genepop-exact-tests, $p < 0.001$). There is thus significant genetic structure in the complete dataset of both *B. minimum* and *B. normannum*.

TABLE 4

Genetic differentiation (F-statistics) in *Bembidion minimum* and *B. normannum*, at various geographic scales (A= regional, i.e. Flanders and southern part of the Netherlands, B= Atlantic, i.e. A + other Atlantic coastal regions, C= Atlantic + Mediterranean region); F_{ST} (WC)= according to Weir & Cockerham; G_{ST} (N) according to Nei, G_{ST} (NC), according to Nei & Chesser.

species	F_{ST} (WC)	$p(F_{ST})$	G_{ST} (N)	G_{ST} (NC)	geographic scale
B. minimum	0.0113	<0.01	0.0295	0.0131	A
B. minimum	0.0346	<0.001	0.0513	0.0353	B
B. normannum	0.0115	<0.01	0.0160	0.0039	A
B. normannum	0.0295	<0.001	0.0288	0.0152	B
B. normannum	0.0565	<0.001	0.0594	0.0453	C

TABLE 5

Significant pairwise exact tests on genetic differentiation between all pairs of populations in *Bembidion minimum*; all population pairs mentioned (pop 1 with each of the mentioned pop 2) are significantly different at p< 0.0033 (Bonferroni-corrected alpha-level); f.e., AUT is significantly different from HUM, MOR, NIE en ZWC for the AO locus; for abbreviations see Table 1.

pop1	pop2									locus
AUT			HUM	MOR	NIE				ZWC	*AO*
CAN	EXE	FER	HUM	MOR	NIE		OSS	SAE	ZWC	*AO*
CAN					NIE		OSS			*MPI*
CAN				MOR	NIE	OOS				*GPI*
SOM	EXE		HUM	MOR	NIE		OSS	SAE	ZWC	*AO*
SOM						OOS				*GPI*
SOM						OOS				*PEPD*
MOR			NIE		OSS					*MPI*
MOR	HOL	HOR	NIE	OOS	OSS	SEV				*AO*
HOL	ZWR									*AO*
HUM	NIE	OSS								*MPI*
SAE	NIE	OSS								*MPI*

TABLE 6

Significant pairwise exact tests on genetic differentiation between all pairs of populations in *Bembidion normannum*; all population pairs mentioned (pop 1 and mentioned pop 2) are significantly different at p< 0.0036 (Bonferroni-corrected alpha-level); for abbreviations see Table 1.

pop1	pop2								locus
ROU		AUT	CAN	GAC	GIR	GUA	GUB	HOL	HUM	*AO*
ROU		AUT	CAN		GIR	GUA		HOL	HUM	*MPI*
ROU		AUT	CAN	GAC				HOL		*GPI*
ROU			CAN	GAC	GIR			HOL		*PGM*
ROU		MOL	MSB	MSG	RYE		ZWC	ZWR		*AO*
ROU		MOL	MSB	MSG		THO	ZWC	ZWR		*MPI*
ROU								ZWR		*GPI*
ROU		MOL	MSB	MSG	RYE	THO	ZWC	ZWR		*PGM*
THO	GIR	MOL	MSB	MSG						*AO*
HOL	GIR	GUA	HUM	ZWC						*PGM*

Geographic scaling and genetic diversity in relation to habitat and population size

Average gene diversities (expected heterozygosities) based on all loci are plotted for all populations of both species against the population size estimates (density x area) (Figs 3-4). Only a very weak and not significant relationship is observed in both species. No significant correlation is thus found between heterozygosities (H_{exp}) of either species and population size. Data on habitat size or saltmarsh age do not improve the significance of the relationships in multiple regressions. Fig. 4 shows that the distinctly larger value found in the Mediterranean sample cannot be explained as a consequence of a higher population size of the species in that particular saltmarsh. Inspection of the allele frequency table (Table 3) shows that a higher heterozygosity in the Mediterranean sample is especially visible at the AO and MPI loci. This higher variability is only to a very low degree caused by unique alleles but results especially from a higher mean number of (non-unique) alleles, occurring at more equal frequencies and thus increasing heterozygosity. In *B. minimum*, the three southernmost samples (Authie, Canche and Somme) also show higher genetic variability scores, especially resulting from slightly more elevated heterozygosities in AO and PEPD.

Genetic differentiation and geographic distance

Both *Bembidion* species show significant genetic differentiation (F_{ST}) between populations (Table 4) at each of the tested geographic scales, even at the regional level. Overall, about 2 to 6 % of the total genetic variation is explained by differentiation between populations. Values derived from different F_{ST}-estimates yield comparable results, although G_{ST} (NEI)-estimates always are somewhat higher. More importantly, genetic differentiation estimates clearly increase at a larger geographic scale (Table 4). There is thus, to some extent, an increased genetic differentiation as a result of increased geographic scale (see later).

UPGMA-dendrograms based on Nei's genetic distance are shown in Fig. 5 for *B. minimum* and Fig. 6 for *B. normannum* (dendrograms based on Rogers' genetic distance yielded similar groupings and are therefore not shown). Detailed results on population differentiation (pairwise
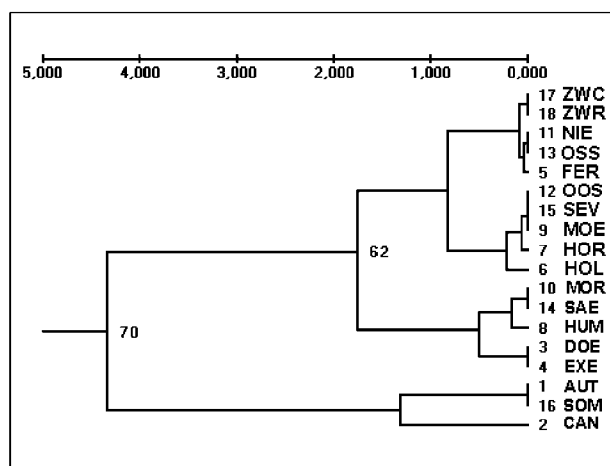

Fig. 5. – UPGMA-dendrogram based on Nei's genetic distance for all studied populations in *Bembidion minimum*; population number and letter codes added; bootstrap-values exceeding 50 added only (first node and second node).
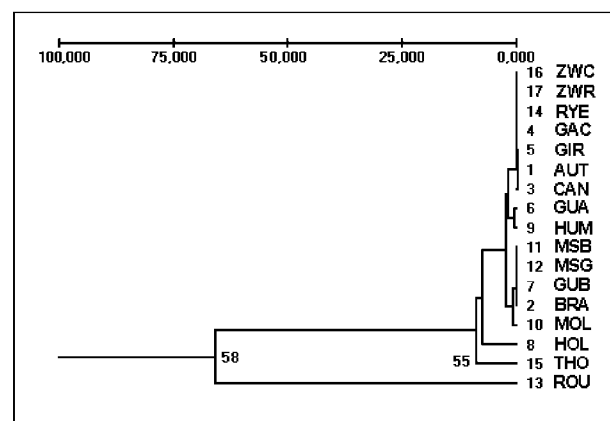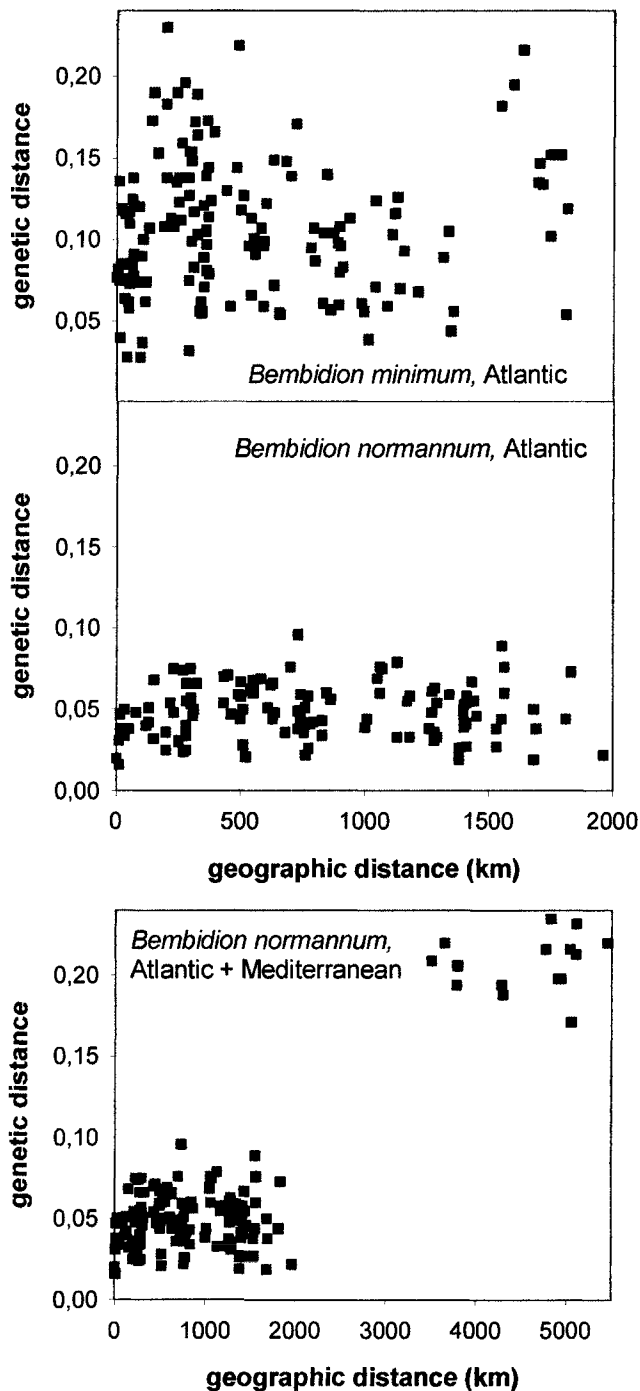

Figs. 3-4. – Genetic diversity (expected heterozygosity) in relation to population size estimates for *Bembidion minimum* and *B. normannum* (see text for further explanation; black square in Fig. 4 refers to the Mediterranean population).

Fig. 6. – UPGMA-dendrogram based on Nei's genetic distance for all studied populations in *Bembidion normannum;* population number and letter codes added; bootstrap- values exceeding 50 added only (first node and second node).
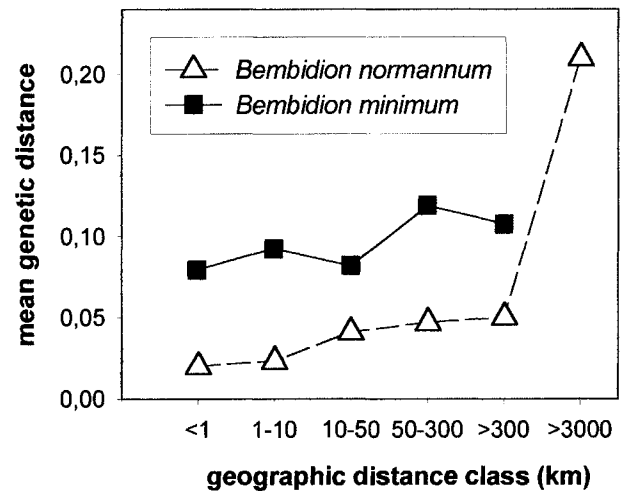
Fig. 10. – Mean genetic distance for various geographic distance classes compared between *Bembidion minimum* and *B. normannum*.

Figs. 7-9. – Pairwise values for genetic distance in relation to geographic distance (km) for: *Bembidion minimum* (Atlantic data only, upper figure), *B. normannum* (Atlantic data only, middle figure) and *B. normannum* (Mediterranean and Atlantic data, lower figure).

exact tests, cf. Tables 5 and 6) show numerous highly significant differences, coinciding with the observed well-supported groups in the dendrograms (cf. relatively high bootstrap-values for basal nodes).

In *B. minimum*, beetles from the Authie, Canche and Somme estuaries are well differentiated from nearly all other marshes. These estuaries concern the three southernmost

effect of isolation by distance at lower geographical scale. This holds true for both species, but again especially for *B. normannum*. At the same time, the comparison between Fig. 7 and 8, as well as the results for both species regrouped in Fig. 10, shows that for a similar geographic scale, genetic differentiation is always somewhat higher for Atlantic *B. minimum* populations than for Atlantic *B. normannum*. This coincides with much lower genetic variability estimates for Atlantic *B. normannum* (Figs 3-4), a much rarer species and more discontinuously distributed in Western Europe than *B. minimum*.

DISCUSSION

Mean gene diversity is larger in the Atlantic populations of *B. minimum* than in the much more discontinuously distributed Atlantic populations of *B. normannum*. For the latter species, the single Mediterranean sample yields a distinctly higher value than do all Atlantic samples. This coincides with a much higher incidence of *B. normannum* in Mediterranean saltmarshes. Indeed, in that area *B. normannum* is one of the most common halobiontic ground beetles occurring in high densities at numerous sites (GAUTIER, 1979; VERDIER & QUÉZEL, 1951). *B. minimum*, on the other hand, appears to be much rarer and less abundant in Mediterranean saltmarshes. Although our result is derived from a relatively low number of loci only, it gives a strong indication that the evolutionary origin of *B. normannum* lies in the Mediterranean area and/or that this area has served as glacial refugium. It is not possible to suggest such a possible centre of origin for *B. minimum* based on our data. We intend to study this question further, if possible by means of additional and more powerful genetic markers, such as microsatellite markers. In an earlier study on two other saltmarsh beetles we concluded that age and size of European saltmarshes, although diffi-

cult to study independently, appeared to be important for the genetic structure of halobiontic beetles (DESENDER et al., 1998). There was, however, not a clearcut linear increase in genetic diversity with population size.

Several European ground beetles, including these *Bembidion* species, are highly specialised halobionts, limited in their occurrence to one or several saltmarsh microhabitats, where relatively high densities (occasionally up to 10-20 individuals per m²) can be reached (THIELE 1977). Effects of genetic erosion in fragmented populations cannot be observed in the studied populations of *B. minimum* and *B. normannum*. The absence of a relation between population or habitat size and genetic diversity indicates that effective population sizes in our study sites still are sufficiently high. Indeed, even in the smallest saltmarshes studied, population estimates of *Bembidion* always exceeded 1000 individuals. It is not excluded that genetic erosion could be observed in other sites where these species occur in lower numbers (e.g. *Bembidion minimum* in Mediterranean populations?). On the other hand, both species possess a high dispersal power. Therefore some gene flow probably still occurs regularly between most populations (at least in *B. minimum* and at a regional scale), counteracting possible temporary losses of genetic variability in small populations.

Nevertheless, we observe for both species significant genetic substructuring (differentiation), indicating at least some influence of geographic scaling and position between at least some of the sites and/or suggesting isolation by geographical distance. Among-population genetic differentiation in two other halobiontic ground beetles in a previous study was highly significant (DESENDER et al., 1998). Especially in the wing polymorphic *Pogonus chalceus* nearly all populations studied appeared to be genetically distinct, based on both allozyme and wing polymorphism data. Even the constantly winged *Dicheirotrichus gustavii* showed numerous statistically significant differences in allele frequencies between pairs of populations (DESENDER et al., 1998). Conserving a maximal genetic diversity for saltmarsh beetles therefore requires the protection of as much of the few remaining sites as possible. Significant genetic substructuring (allozymes) has been reported already for many insects, including beetles (HSIAO, 1989; KING, 1987, KNOLL et al., 1996)

Estimates of genetic differentiation in the present study are lower than the mean values (F_{ST} = 0.103) obtained for 30 other beetle species (HSIAO, 1989), which are known to be among the highest recorded for insects (WARD et al., 1992). Theories that relate variation in F_{ST} to variation in rates of gene flow indeed predict that species with a high dispersal power should show less population structuring (WAPLES, 1998; WARD et al., 1992). Empirical results for two ground beetle species with a supposedly low dispersal potential and occurring in heathland fragments did not follow this prediction (DE VRIES, 1996). Only low levels of population substructuring were observed and gene flow between populations appeared to be difficult to estimate in

a fragmented landscape without additional data on dispersal. Differences between both *Bembidion* species (differentiation at a similar geographical scaling is somewhat higher in *B. minimum* as in *B. normannum*) might also be due to unequal dispersal power or flight behaviour. In the future we will study the morphology of flight muscles in time-series of both species in order to look for possible differences in the seasonal occurrence of functional flight musculature in conjunction with reproduction. A hypothesis to be tested is that less flight activity (gene flow) would be expected to occur in *B. minimum* than in *B. normannum*.

Although *B. minimum* and *B. normannum* are considered highly mobile, they are readily affected by the current state of isolation. At the moment, effects of isolation between *B. minimum* and *B. normannum* populations only appear at a relatively large geographical scale. Mediterranean *B. normannum* are highly significantly differentiated from Atlantic populations. Atlantic beetles of another saltmarsh species, *Pogonus chalceus*, were also genetically distinct from Mediterranean populations (DESENDER & SERRANO, 1999), while genetic diversity was not distinctly higher in the Mediterranean area. With further disappearance of saltmarshes or further decrease of saltmarsh habitat quality in the future, isolation between extant *Bembidion* populations is expected to increase as gene flow could become more limited, especially for the rarer *B. normannum*. As size and age of saltmarshes does not seem to be of major importance for the genetic constitution of these species, the maintenance of small and even young salt marshes could already be a good choice for maintaining sufficient genetic variation and stable populations of both species. Creation of new, even small, saltmarshes is expected to be positive for the protection of these *Bembidion* species. However, we have to consider that such nature development actions may be positive for both *Bembidion* species, but not sufficient for other, less mobile, saltmarsh beetles. An example is the halobiontic *Pogonus chalceus*, which showed brachyptery and a low dispersal power in old and isolated saltmarshes (DESENDER & SERRANO, 1999). DESENDER et al. (1998) already came to the conclusion that small and recent saltmarshes are nevertheless very important in the long term survival of *Pogonus chalceus* populations too, but for a different reason. Indeed, such sites appeared to be the only ones left with populations of macropterous individuals (capable of dispersal by flight and thus of (re)colonisation). Overall, such (recently established) populations thus are expected to contribute substantially in a well-functioning metapopulation structure. In this way, long term survival of the species would be much increased (HASTINGS & HARRISON, 1994). Genetic results, as obtained in our study, suggest that metapopulations probably function at a relatively large and at least regional scale in both *Bembidion minimum* and *B. normannum*. More genetic data, especially with more and powerful markers, are needed to confirm this hypothesis.

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