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Genetic population structure of the Postglacial relict diving beetle *Hydroporus glabriusculus* Aubé (Coleoptera: Dytiscidae)

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The genetic population structure of the diving beetle *Hydroporus glabriusculus* was investigated by studying electrophoretic variation at nine enzyme loci. *H. glabriusculus* is a boreal Palaearctic dytiscid occurring in the far north of Eurasia and as scattered colonies elsewhere in western and central Europe, where it is considered to be a remnant of the early Postglacial biota. Analysis of allele frequency variation revealed considerable population substructuring within localities, which was supported by the field observation that the species exists as small, discrete colonies within a fen habitat showing considerable micro-ecological variation. This appears to be the first time such a demic population structure has been reported in a fully aquatic freshwater organism.

Keywords: demes, *Hydroporus,* microgeographic substructuring, population genetics.

Introduction

Population substructuring on a microgeographic scale has been demonstrated in a wide variety of organisms, showing that gene flow is often extremely limited, even over relatively short distances (e.g. Selander & Ochman, 1983; Selander & Whittam, 1983). Spatial or temporal differences in reproductive activity can lead to the existence of separate populations in the same geographical area (e.g. Allendorf *et al.,* 1976). Local inbreeding results in marked population substructuring in a number of organisms, among the best studied of which are rodents (e.g. Selander, 1970; Berry, 1978; Patton & Feder, 1981; Chesser, 1983). In many invertebrates a combination of limited dispersal ability, and microhabitat fidelity results in marked population subdivision, sometimes over small geographical distances (e.g. Janson & Ward, 1984; King, 1987; Crouau-Roy, 1988).

Few studies of the population genetics of aquatic invertebrates have been conducted, with most concentrating on species of rivers or large ponds and lakes (e.g. Jarne & Delay, 1990; Seigsmund & Muller, 1991). The insect examined here is restricted to primary fen habitats which show considerable micro-ecological variation. Such a situation would be predicted to result in population substructuring in a small invertebrate with restricted ecological tolerance and limited dispersal ability. As discussed below, this is indeed the case with *Hydroporus glabriusculus.*

H. glabriusculus is a small predaceous water beetle belonging to the family Dytiscidae (diving beetles). *Hydroporus* species are 2–9 mm in length, and rather similar in general appearance. *H. glabriusculus* is readily distinguished from other *Hydroporus* by the characteristic penis of the male (Sinclair, 1975).

The present world distribution of the species is centred on the northern forest and tundra belt of Eurasia. *H. glabriusculus* is found widely in Scandinavia, particularly in the north (Nilsson & Persson, 1989), and ranges through Finnish Lapland and the Kola Peninsula to much of northern and central Siberia, east to Yakutia and south to Novgorod and Transbaikalia (R. B. Angus, personal communication; A. N. Nilsson, personal communication; Zaitzev, 1972). The species has in the past been reported from the Nearctic region (e.g. Larson, 1975), but work by Sinclair (1975) has shown that the taxon known as *H. glabriusculus* in North America is a separate undescribed *Hydroporus.*

In addition to being widespread in the far north of Eurasia, *H. glabriusculus* is found further south in

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western Europe, in the British Isles (Sinclair, 1975; Foster, 1982; Bilton, 1988), Germany (Schaeflein, 1979; Braasch, 1989), Poland (Galewski, 1971), Denmark (R. B. Angus, personal communication) and Holland (B. Drost, personal communication). In these areas it occurs as isolated colonies in small regions of mesotrophic and eutrophic fen whose development usually dates back to the early Postglacial. These localities are invariably of high conservation value, being home to a range of plants and invertebrates which are endangered throughout the region (Schaeflein, 1979; Foster, 1989; Bratton, 1991). Although difficulties in the recognition of subfossil fragments have prevented the identification of *H. glabriusculus* in fossil insect assemblages, the biotopes and invertebrates with which the species is associated in its southern sites were certainly more widespread in the early Flandrian (e.g. Girling, 1977; Kerney, 1977; O'Connel, 1980). For this reason, coupled with its restriction to ancient wetlands, *H. glabriusculus* is generally considered to be a Postglacial relict species in western Europe south of Scandinavia (Schaeflein, 1979; Foster, 1982).

Materials and methods

Localities and sampling

Specimens of *Hydroporus glabriusculus* used in the present study were obtained from the following localities.

England; Norfolk: Thompson Common (TL 93-96-), East Harling Common (TL 99-87-), Foulden Common (TF 76-00-), East Walton Common (TF 73-16-), Lambs Common (TF 71-17-). All these sites consist of series of small fens developed in hollows created by the collapse of ice lenses in permafrost (Sparks *et al.*, 1972). These have been likened to the much larger periglacial pingos. At each locality beetles were taken from a single pingo fen only.

Ireland; Co. Westmeath: Scragh Bog (N 42-59-), Walshestown Fen (N 39-54-).

Scotland; Roxburgh: Lang Moss (NT 50-29-).

Sweden; Umeå district: Bog nr Nebre Mesele, Lat. 64° North (coll. A. N. Nilsson).

At each site approximately 30 individuals were taken for use in allozyme electrophoresis. The rather low abundance of this species made the collection of larger samples impossible.

Sampling methods and specimen care

Adult beetles were collected on a single visit to each site, using a D-framed pond net. Specimens utilized were caught during the period April–June. The insects were transported alive to the laboratory in containers filled with damp moss, after which they were maintained in an outdoor insectary.

Electrophoretic methods

Sample preparation. Preliminary studies showed that satisfactory levels of enzyme activity could only be obtained by using fresh material of *Hydroporus glabriusculus*. Beetles were killed by removing the head with watchmakers' forceps, and then homogenized in 0.1 ml of a solution 0.1 per cent NADP, 0.05 per cent β-mercaptoethanol and 0.1 per cent Triton X-100. The resulting suspension was spun at 15,000 ***g*** for 15 min in a Beckman microcentrifuge maintained at 4°C. After centrifugation the supernatant was placed on ice and used immediately in electrophoresis.

Electrophoresis and enzyme staining. The small adult size of the insect studied severely restricted the sample volume obtained from a single individual. For this reason cellulose acetate gels were used in electrophoresis because they allow the resolution of specific enzyme systems from very small (sub-microlitre) quantities (Easteal & Boussy, 1987). Cellulose acetate has a number of other advantages over more traditional media, which are fully discussed by Richardson *et al.* (1986).

A total of 19 enzyme systems was screened in the present study. Of these 12 (alcohol dehydrogenase, amylase, fumerate hydratase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, glycerol-3-phosphate dehydrogenase, hexokinase, hydroxybutyrate dehydrogenase, iditol dehydrogenase, leucine amino peptidase, 6-phosphogluconate dehydrogenase and xanthine dehydrogenase) failed to stain reliably, and were rejected. This left seven enzyme systems (esterases, phosphoglucomutase, glucose phosphate isomerase, isocitrate dehydrogenase, malic enzyme, lactate dehydrogenase and mannose phosphate isomerase), with nine scoreable loci, which were used in genetic analysis. The running conditions for these are listed in Table 1. The buffers used when running gels were as follows. Tris glycine, 0.02 M tris; 0.2 M glycine pH 8.0; 2/5 Tris citrate, 0.04 M tris to which 0.07 M citrate was added until pH 7.6. Staining followed Easteal & Boussy (1987) and Richardson *et al.* (1986). Visualization of esterases was best achieved using an α-naphthyl acetate substrate.

Statistical methods. The following were calculated for each locality. Mean number of alleles per locus, percentage of loci polymorphic (95 and 99 per cent) and mean heterozygosity.

Population structure was analysed using *F*-statistics (Nei, 1977; Wright, 1965, 1978). In the dicussion

Table 1 Running conditions for enzymes studied. For details of buffer systems see text. All enzymes run at 200 V

Enzyme system	Run buffer	Run time (min)
Esterase (EST)	tris-glycine	30
Phosphoglucomutase (PGM)	tris-glycine	15
Glucose phosphate isomerase (GPI)	2/5 tris citrate	30
Isocitrate dehydrogenase (IDH)	2/5 tris citrate	45
Malic enzyme (ME)	2/5 tris citrate	30
Lactate dehydrogenase (LDH)	2/5 tris citrate	15
Mannose phosphate isomerase (MPI)	2/5 tris citrate	30

which follows the term 'population' refers to all material of *H. glabriusculus* examined, and 'subpopulation' to beetles from a single locality, following Wright (1978). For each polymorphic locus the three statistics F_{is}, F_{it} and F_{st} were calculated, firstly within regions, and then over all samples. F_{it} represents the correlation between gametes that unite to produce individuals relative to the gametes of the total population. F_{is} is the average over all subdivisions of the correlation between uniting gametes relative to their own subdivision. It thus represents an inbreeding coefficient for each subpopulation, and is therefore equivalent to the fixation index (Wright, 1978). Both F_{it} and F_{is} are measures of deviation from panmixis, becoming positive with homozygote excess, and negative under heterozygote excess. The significance of the deviations of F_{is} from zero was tested using the formula,

$$n(F_{is})^2$$

where n is the total number of individuals, in a chi-squared distribution with one degree of freedom, as detailed by Baker (1981) and Patton & Feder (1981).

F_{st} is interpreted by Wright (1978) as a measure of the amount of differentiation amongst subpopulations, relative to the limiting amount under complete fixation. When all subpopulations are fixed for separate alleles at a locus F_{st} becomes 1. F_{st} is zero when subpopulations possess identical gene frequencies. The significance of the gene frequency differences between localitites was elucidated using the chi-square test,

$$\chi^2 = 2NF_{st}(k-1)$$

with $(k-1)(s-1)$ degrees of freedom. In this formula N is the total sample size, k is the number of alleles per locus and s is the number of localities (Eanes & Koehn, 1978).

Linkage disequilibrium was calculated between polymorphic (95 per cent) loci using the chi-squared test. With the exception of *EST-3* all loci were approximated to two-allele systems by summing the frequencies of rarer alleles.

Results and discussion

Enzyme phenotypes

In addition to those scored, extra zones of activity were observed on gels stained for esterases (two zones), phosphoglucomutase (one zone) and glucose phosphate isomerase (one zone), which presumably represent other, unscoreable loci. Alleles were labelled alphabetically in order of increasing electrophoretic mobility. An interesting feature of the isozymes examined is that, with one possible exception, they all appear to have monomeric products; heterozygotes producing a two-banded phenotype. In the case of isocitrate dehydrogenase it is impossible to comment on the quaternary structure because heterozygotes were never observed.

Enzyme phenotypes had to be scored from the gels immediately after staining, because after drying the zones of activity were no longer visible, particularly in the case of the more weakly staining polymorphic enzymes. This is probably a consequence of working with low volumes of sample of limited activity.

Allele frequencies in H. glabriusculus

Table 2 presents allele frequencies at all loci for the nine populations investigated. Eight showed some variation within and between populations, *EST-1* being monomorphic in all localities. The polymorphic loci investigated showed considerable differences in their levels of variability. In the case of *PGM-1* and *IDH-1*, the frequency of the most abundant allele never dropped below 0.8. Indeed *PGM-1* was fixed for a single allele in eight out of nine localities, showing variation only in the sample from Scragh Bog. At the other extreme the highly polymorphic *EST-3* locus was always represented by at least three alleles, the frequency of the most abundant allele never surpassing 0.55. For most other loci the pattern of variation was similar, a number of localities being fixed for a single

Table 2 Summary of allele frequencies at all loci. Numbers at the head of the table refer to localities: 1 = Thompson Common; 2 = East Walton Common; 3 = Foulden Common; 4 = Lambs Common; 5 = East Harling Common; 6 = Walshestown Fen; 7 = Umeå; 8 = Lang Moss; 9 = Scragh Bog

Locus		1	2	3	4	5	6	7	8	9
	(*n*)	33	33	33	33	33	32	32	33	32
EST-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-2	A	0.00	0.00	0.00	0.00	0.00	0.016	0.00	0.015	0.063
	B	0.00	0.00	0.00	0.00	0.00	0.016	0.031	0.030	0.031
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.045	0.094
	D	1.00	1.00	1.00	1.00	1.00	0.969	0.969	0.909	0.813
EST-3	A	0.015	0.00	0.00	0.00	0.00	0.00	0.031	0.030	0.094
	B	0.045	0.015	0.00	0.00	0.015	0.00	0.016	0.045	0.031
	C	0.242	0.152	0.152	0.242	0.212	0.516	0.063	0.242	0.484
	D	0.364	0.409	0.545	0.439	0.530	0.469	0.500	0.318	0.328
	E	0.333	0.424	0.303	0.318	0.242	0.016	0.391	0.364	0.063
PGM-1	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.172
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.828
GPI-2	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00
	B	1.00	1.00	0.985	1.00	1.00	0.953	0.953	0.803	0.656
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.031	0.167	0.328
	D	0.00	0.00	0.015	0.00	0.00	0.047	0.016	0.00	0.016
IDH-1	A	1.00	0.985	1.00	1.00	1.00	1.00	0.938	0.909	0.813
	B	0.00	0.015	0.00	0.00	0.00	0.00	0.00	0.00	0.063
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.063	0.091	0.125
ME-1	A	0.00	0.00	0.00	0.00	0.00	0.063	0.016	0.136	0.047
	B	0.076	0.00	0.00	0.00	0.00	0.078	0.00	0.212	0.063
	C	0.924	1.00	1.00	1.00	1.00	0.859	0.938	0.621	0.781
	D	0.00	0.00	0.00	0.00	0.00	0.00	0.047	0.030	0.109
LDH-1	A	0.00	0.00	0.00	0.00	0.00	0.047	0.00	0.00	0.00
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.016
	C	0.00	0.00	0.00	0.00	0.00	0.016	0.00	0.333	0.047
	D	1.00	1.00	1.00	1.00	1.00	0.938	1.00	0.667	0.938
MPI-1	A	0.045	0.00	0.00	0.00	0.030	0.438	0.188	0.091	0.156
	B	0.955	1.00	1.00	1.00	0.970	0.563	0.688	0.909	0.656
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.125	0.00	0.188

(*n*) indicates sample sizes.

allele, with additional alleles present at lower frequencies elsewhere.

Levels of genetic variability within populations

Table 3 presents the following measures of genetic variability; mean number of alleles per locus, the percentage of loci polymorphic (95 and 99 per cent levels), and mean heterozygosity per locus (direct count measure). No distinction can be made between the relict populations of this insect in the British Isles and the population from Sweden on the basis of allele frequencies. There is, however, a clear difference between the Norfolk localities (1–5), with comparatively low levels of variability, and other samples. This reduced genetic variability is considered to have resulted from the bottlenecking of populations ancestral to those present on the Norfolk Breck during the Lateglacial (Bilton, 1991).

In Norfolk both the mean number of alleles per locus and the percentage of loci polymorphic appear to be related to locality size. The largest Norfolk station sampled was the fen at Thompson Common, and here *H. glabriusculus* shows a higher level of genetic variation than elsewhere in the region. Other Breckland localities contain populations with remarkably similar low levels of variability, the sample from the pingo fen at Lambs Common being the most depauperate. A similar pattern emerges when all sites are considered together, Scragh Bog, the largest locality sampled, providing the most variable population of *H. glabriusculus* encountered. As the level of genetic variation in a

Table 3 Measures of genetic variability within each locality

Locality	Sample size per locus (n)	Mean number alleles/locus	Loci polymorphic* (%)	Mean heterozygosity/ locus†
1. Thompson	33	1.7 (0.4)	22.2/33.3	0.054 (0.047)
2. E. Walton	33	1.4 (0.3)	11.1/22.2	0.064 (0.060)
3. Foulden	33	1.3 (0.2)	11.1/22.2	0.071 (0.067)
4. Lambs	33	1.2 (0.2)	11.1/11.1	0.037 (0.037)
5. E. Harling	33	1.4 (0.3)	11.1/22.2	0.061 (0.061)
6. Walshestown	32	2.1 (0.3)	44.4/66.6	0.122 (0.071)
7. Umeå	32	2.3 (0.4)	44.4/66.6	0.090 (0.054)
8. Lang Moss	33	2.7 (0.5)	77.7/77.7	0.104 (0.063)
9. Scragh bog	32	3.1 (0.4)	88.8/88.8	0.188 (0.041)

Standard errors are given in parenthesis.
*0.99/0.95 per cent levels, respectively.
†Direct-count measure.

population is largely a product of the mutation rate and effective population size (Kimura, 1983), larger populations would be expected to contain higher levels of endogenous variation. Although nothing is known with regard to population size at the sites investigated, it is very likely that larger areas of habitat support more individuals.

The percentage of loci polymorphic (95 per cent level) has a mean value of 30 per cent when all samples are pooled. This value is in accordance with much of the existing data for insects and other invertebrates (e.g. Selander, 1976; Nevo, 1978; Wright, 1978). The mean value is, however, determined to a large extent by the low frequency of polymorphic enzyme loci in the Norfolk populations. When these samples are excluded from the analysis a mean value of 74.9 per cent is obtained for the remaining populations. Such a figure may be a better reflection of the level of enzyme variation present in populations of *H. glabriusculus* in most of its range because the colonies on the Norfolk Breck appear to represent a special case, where variability has been reduced due to periods of low population size during the Pleistocene (Bilton, 1991).

Within-locality population structure

Values of F_{is} in Table 4 testify to the existence of highly significant deviations from Hardy–Weinberg expectations, largely in the form of heterozygote deficiencies. This can result from inbreeding (Wright, 1978), or from the pooling of subpopulations which differ in allele frequency (Wahlund effect — Hartl, 1980; Spiess, 1989). No information is available concerning mate choice in this species, or for any other dytiscid, so the possibility of some form of non-random mating system, based on degrees of relatedness, cannot be entirely discounted. Under the influences of inbreeding alone, however, values of F_{is} would be expected to be identical across all loci (Wright, 1978). The values of F_{is} in Table 4 differ between loci when considered within regions. This could result from natural selection acting independently on individual loci (Wright, 1978). Additional evidence to suggest a role for natural selection in controlling the patterns of variation is lacking, however, and observations on the ecology of *H. glabriusculus* suggest that population substructuring is the major factor responsible for the deviations from equilibrium frequencies observed (see below).

There is good evidence to suggest that the Wahlund effect is responsible for the F_{is} values observed. This study was conducted under the assumption that each locality contains a single panmictic population of *H. glabriusculus*. The spatial distribution of the beetle within sites, however, is strongly indicative of the existence of a further demic structure. Individuals were found to be aggregated in small pockets of habitat (usually flooded carpets of *Drepanocladus* and *Acrocladium* mosses), each containing in the order of 5–15 specimens, surrounded by areas in which no beetles could be found (D. T. Bilton, unpublished observation). The spatial separation of these clusters varied with locality, but distances between them were typically below 10 m with the exception of Irish localities (see below). Beetles from different clusters within a locality were pooled during the electrophoretic study. If these aggregates are small subpopulations differing to some extent in allelic frequency, their pooling during analysis would result in a Wahlund effect. This does not imply high levels of genetic differentiation between subpopulations (Crouau-Roy, 1988), nor does it indicate that the subpopulations have been separated for many generations. A Wahlund

Table 4 Summary of *F*-statistics

Localities	Locus	Number of alleles	F_{is}	F_{it}	F_{st}
Norfolk	*EST-3*	5	0.227**	0.239	0.016 ns
(five sites)	*GPI-2*	2	−0.015 ns	−0.003	0.012 ns
	IDH-1	2	−0.015 ns	−0.003	0.012 ns
	ME-1	2	0.784***	0.797	0.062***
	MPI-1	2	0.792***	0.797	0.025 ns
Mean			0.268***	0.282	0.018**
Ireland					
(two sites)	*EST-2*	4	0.677***	0.691	0.044***
	EST-3	5	0.245 ns	0.255	0.014 ns
	PGM-1	2	−0.208 ns	−0.094	0.094***
	GPI-2	3	0.716***	0.759	0.152***
	IDH-1	3	1.000***	1.000	0.079***
	ME-1	4	0.248 *	0.259	0.015 ns
	LDH-1	4	0.473***	0.477	0.007 ns
	MPI-1	3	0.938***	0.941	0.058***
Mean			0.527***	0.553	0.054***
All regions	*EST-2*	4	0.700***	0.721	0.070***
(nine sites)	*EST-3*	5	0.266***	0.316	0.068***
	PGM-1	2	−0.208***	−0.019	0.156***
	GPI-2	4	0.751***	0.797	0.183***
	IDH-1	3	0.952***	0.956	0.081***
	ME-1	4	0.248***	0.342	0.125***
	LDH-1	4	0.817***	0.857	0.221***
	MPI-1	3	0.808***	0.847	0.199***
Mean			0.463***	0.527	0.199***

*$P<0.05$,**$P<0.01$,***$P<0.001$.
ns = not significant.

effect was detected in migratory samples of the monarch butterfly (*Danaus plexippus* L.) which contain populations from all over the western US which had been isolated from each other for a maximum of four generations (Eanes & Koehn, 1978). *H. glabriusculus* appears to be rather sedentary (D. T. Bilton, unpublished observation), and lives in wet vegetation rather than open water during most of the life cycle (D. T. Bilton, personal observation), factors which are probably important in the maintenance of semi-isolated demes in this species.

Population substructuring over limited geographical areas has been reported in a number of animal groups, including salmonid fish (Allendorf *et al.*, 1976), rodents (Selander, 1970; Berry, 1978; Patton & Feder, 1981; Chesser, 1983) molluscs (Selander & Ochman, 1983; Janson & Ward, 1984), and some beetles (King, 1987; Crouau-Roy, 1988).

Mean F_{is} values for Norfolk and Irish localities are also presented in Table 4. Although both these values show significant homozygote excesses, the mean F_{is} in the Irish sites is roughly twice that observed in Norfolk. Again this supports the existence of a Wahlund effect within the samples. At both Irish stations beetles were sampled from a much wider area than was covered in Norfolk, clusters being separated by up to 50 m. Such widely spaced subpopulations would be expected to show greater differences in gene frequencies, due to lower levels of movement between them, compared with those which are closer together.

F_{it} values for all loci are also given in Table 4. With only four exceptions F_{it} values demonstrate an excess of homozygotes within the total sample of *H. glabriusculus*. This results from the pooling of populations with differing alleleic frequencies, in an identical way to the Wahlund effect proposed above to account for the positive F_{is} values recorded within each sample.

Significant associations between alleles at different loci are shown in Table 5. Such linkage disequilibrium can result from a number of factors (Hartl, 1980), only one of which is the physical linkage of genes on the same chromosome. The data presented here are insufficient to make any conclusions regarding physical linkage on the basis of invariant associations between

Table 5 Pairs of loci showing significant linkage disequilibrium within localities. Associations detected using the chi-square test

Locality	Loci	Chi-square
6. Walshestown	*ME-1/LDH-1*	12.26***
7. Umeå	*IDH-1/MPI-1*	9.68**
8. Lang Moss	*GPI-2/LDH-1*	7.61*
	ME-1/MPI-1	24.16***
9. Scragh Bog	*EST-2/LDH-1*	9.679**
	EST-3/PGM-1	13.973**
	PGM-1/GPI-2	18.00***
	PGM-1/MPI-1	18.00***
	GPI-2/ME-1	34.96***
	GPI-2/MPI-1	13.498**
	IDH-1/ME-1	13.316**
	IDH-1/MPI-1	6.951*
	ME-1/MPI-1	15.956**

*$P<0.05$,**$P<0.01$,***$P<0.001$.

localities. Linkage disequilibrium of this type could also be a result of the existence of subpopulations with differing allelic frequencies at two or more loci (e.g. Allendorf *et al.*, 1976). Since there is good evidence for the existence of separate demes within a locality this could be used to explain the observed linkage disequilibrium.

Inter-locality population differentiation

F_{st} values in Table 4 were first calculated within regions which contained more than one sampled population (Norfolk & Ireland), and then for all the localities as a whole.

Overall genetic differentiation levels (mean F_{st} over all polymorphic loci) show that in Norfolk 1.8 per cent of the total variance of allele frequencies was due to genetic differentiation between populations, the remaining 98.2 per cent of the genetic diversity being accounted for by variation amongst beetles within a locality. For those Irish sites analysed these values are 5.4 and 94.6 per cent, respectively, whilst for all localities combined they are 19.9 and 80.1 per cent. Within regions, limited genetic divergence appears to have occurred between populations, most of the heterogeneity being accounted for by differentiation between the four separate areas (Ireland, Scotland, Norfolk and north Sweden) included in the analysis. However the low values of F_{st} within Norfolk, partly result from three of the polymorphic loci analysed (*GPI-2, IDH-1* and *MPI-1*) being fixed for identical alleles in four, four and three populations respectively (see Table 2 and discussion above). Measures of differentiation between the localities in this area may, therefore, underestimate the degree of isolation of individual populations, all of which now occur in habitat islands, surrounded by inhospitable agricultural land.

The F_{st} values reported here indicate significant differentiation most of which is between regions. This is exactly what would be predicted for a species living in isolated habitat islands between which there is limited migration. Relatively high levels of differentiation have been documented for many other animal species whose distribution is based on isolated areas of habitat (e.g. Gooch & Hetrick, 1979; King, 1987; Crouau-Roy, 1988), or small breeding units between which there is limited genetic exchange (e.g. Selander, 1970; Chesser, 1983). The situation for more widespread species, in which there are apparently high levels of gene flow, is quite different. Eanes & Koehn (1978), for example, report F_{st} as low as 0.009 for monarch butterflies in the eastern U.S.A. The value of this statistic for human populations worldwide (quoted in Nei, 1986) is 0.148, which is somewhat lower than that reported here for *H. glabriusculus* populations in western Europe.

Conclusions

Available genetic evidence suggests that *H. glabriusculus* populations are subdivided into small demes between which there is limited gene flow. These demes probably correspond to the clusters of individuals observed in nature, where the species forms small colonies within a fenland environment which is highly structured on a microgeographic scale. Personal observations show that *H. glabriusculus* is usually an inhabitant of wet moss and vegetation, amongst which it is rather sedentary. Movement between demes in patches of vegetation would only occur at significant levels when periods of high water within sites enable beetles to swim from one colony to another. The current study was conducted after a succession of three particularly dry years in the British Isles, when water-levels at all localities appear to have been abnormally low. This may explain why population structuring was observed to some degree at all sites.

Microgeographic differentiation of the type observed here can result from the action of at least two factors; genetic drift, and natural selection varying between demes. In the present study subpopulations were mixed from very limited areas, the most widely spaced of which were around 50 m apart (Scragh Bog). Areas inhabited by different subpopulations within a site showed no obvious ecological differences, making

it difficult to imagine selection playing a major role in creating gene frequency differences amongst demes. Semi-isolated subpopulations will diverge genetically through the process of random sampling drift, something which is opposed by even limited amounts of migration between demes. In the case of this insect, dispersal between demes has evidently been very low for a number of generations, leading to the differentiation observed.

Population structuring on such a microgeographic scale has been observed in a number of organisms. Some, such as many molluscs, have very limited powers of dispersal, and lead rather sedentary lives. Most genetic studies of aquatic invertebrates show little evidence for population substructuring because in most cases the animals disperse at random within the water body (e.g. Jarne & Delay, 1990). A remarkably similar situation to the one discussed here has been described in semi-aquatic water striders (*Gerris* spp.) which inhabited ponds some tens of metres apart on an open bog surface (Varvia-Aho & Pamilo, 1981). Marked levels of genetic differentiation were discovered, F_{st} values being in the order of 0.02–0.2.

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